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EDITOR'S VIEWPOINT

Shafer (p. 4) found no essential difference in healing of extraction wounds in cortisone-treated and untreated rats until the tenth day. Retardation in epithelization and in sequestration and residual inflammation were observed then in the cortisone-treated animals. Although, as yet, these observations cannot be extended to the human being, if they do prove applicable the early stages of extraction wound healing may lull the clinician into a false feeling of security which may later be shattered by late infection of ulcerated wounds. The findings should be carefully evaluated by postextraction studies in cortisone-treated men and women.

Swartz and Phillips (p. 12) investigated the effect of four different methods for condensing amalgam on the residual mercury contents. Their preliminary results indicate that if a carefully standardized condensation procedure is accompanied by a sound technic for removal of excess mercury, the residual mercury content will be well within practical limits.

Bernick, Warren, and Baker (p. 20) have examined decalcified sections of carious dentin under the microscope. Their observations confirm and extend the concept of four zones in carious dentin. They confirm the concept of hyalinization and calcification of the protoplasmic extensions of odontoblasts with production of the so-called transparent zone of hypercalcified dentin. From their study, it also appears that proteolysis follows decalcification, a finding previously proposed on the basis of chemical and histochemical studies. As methods are refined and interpretations standardized, we should increase our knowledge of oral disease with the aid of this research tool.

Elliott and Pigman (p. 27) were able to show extensive carious development in hamsters fed cariogenic (high sucrose or sucrose and starch) diets, as others had. When they used a human infant diet high in carbohydrate, they found much less caries. It must be recognized that the diets differed not only in type of carbohydrate, but in other factors. Further definite studies are needed to ascertain more certainly the relative cariogenic properties of diets usually consumed by man.

Muhler, Nebergall, and Day (p. 33) report on the effects of various fluorides in reducing enamel solubility and in prevention of rat caries. They point out that the level of fluorine in the drinking water, or stored in the skeleton, does not provide an accurate basis for estimation of protection from caries, but fall back in their discussion to protective statements on the clinically unsupported in vitro findings in the relationship of pH of fluoride solutions to caries reduction. They present strong support for stannous fluoride as a superior compound to sodium fluoride. If human clinical studies bear out these predictions, a worth-while advance will have been offered.

Fisher, Muhler, and Wust (p. 50) found that calcium fluoride formed more rapidly on, or in, powdered enamel treated with potassium fluoride at lower pH; and less rapidly but more deeply with sodium silicofluoride and increasingly at lower pH; but that calcium fluoride did not form under s o d i u m monofluorophosphate treatment. Stannous fluoride treatment produced an amorphous structure but only after prolonged treatment. The authors suggest that both tin and fluoride act in this change. One must hesitate in making clinical transformation of these studies on powdered enamel, remembering the unfortunate results of the use of other clinically untested agents in an effort to reduce dental caries activity.

Turner, Scribner, and Bell (p. 55), studying the relationship between pH, titratable acidity, titratable alkalinity, and caries occurrence, found significant relationships between titratable alkalinity and caries. They point out that this relationship is more evident when caries-free individuals are compared with those with rampant caries. This is not surprising if one considers dental caries a disease in which

bacterially produced acids attack teeth with certain influencing factors present. Whether one has little decay or much decay he has the disease, and there may be factors which modify but do not arrest the process. The single consideration of pH and even of buffering capacity, has not been fruitful, but more precise studies on well-differentiated groups may bear further fruit.

Hess and Lee (p. 62), comparing their own analysis, were unable to find any difference in the protein composition of dentin from carious and noncarious teeth. However, the uninvolved enamel of carious teeth had quite different protein from enamel of noncarious teeth. No explanation is offered, and the question of whether this difference is brought about by caries or is a pre-existing difference is left unanswered. The finding is significant enough to suggest the need for more intensive study.

Fulton and Price (p. 65), pointing out that most studies on eruption and caries attack are of cross-sectional type, that is, based on data from observations on different individuals in given age groups, evaluated data on 100 children followed for at least nine years. By their methods, differences from straight-line developments for age curves of eruption and carious lesions were observed in about 80 per cent of the children, some 20 per cent showing marked deviations. Cross-sectional studies tend to mask such plateaus. The tendency of teeth to erupt in "batches" is an important factor in this plateauing. The early erupting first molars account for 86 per cent of all attacked permanent teeth at 10½ years of age. Some interesting data showing the value of dental treatment in preserving teeth are presented.

Baume, Becks, Ray, and Evans (pp. 80, 91, and 104) report studies on hormonal control of tooth eruption in three papers. In the first, they demonstrate that thyroidectomy reduces tooth eruption rate and, to a lesser degree, tooth size in the rat. Reduced vascularity and effects on epithelium were evidenced. Growth hormone increased eruption rate and tooth size slightly, and bone growth markedly; thyroxin had a greater effect on eruption rate, but calcification remained deficient; while growth hormone and thyroxin together improved histologic aspects to near normal, but did not have as great an effect on growth rate or tooth size. Their second experiment was based on hypophysectomy. Removal of the pituitary, of course, effects the other endocrine glands. Eruption rate again was retarded; the size of the teeth was found to be effected in two weeks, and apical folding occurred followed by disturbance of enamel formation and of odontoblastic activity. In older animals the unfolded incisors maintained a minimal eruption rate. A specific pituitary effect above that of reduced thyrotropic hormone is suggested.

The third study of Baume, Becks, and Evans (p. 104) on the specificity of response to growth hormone and thyroxin in hypophysectomized rats helped to disclose that the pituitary has a more direct and severe effect on eruption than has the thyroid. This reflects the effect of growth hormone as a stimulus to dental and alveolar growth. Thyroxin, on the other hand, stimulates eruption and tooth size, but not alveolar growth. A synergistic effect was noted when both hormones were used in hypophysectomized animals. These basic studies show that growth hormone stimulates the growth processes, and thyroid hormone controls maturation. The results of these studies may not affect clinical or preventive procedures for many years, but they are fundamental.

Nutlay, Bhaskar, Weinmann, and Budy (p. 115) found that estrogens did not change the periodontium of young rats and, in young mice, resulted in reduction in marrow space size by endosteal bone formation only when given over long periods of time (twenty-five to forty days). By contrast, old mice showed widening of the periodontal membrane, resorption of interdental and interradicular bone, and apical proliferation of the epithelial attachment with later pocket formation. Age of the animal seems to affect markedly the ability of the periodontium to maintain itself under adverse systemic action. While interpretation of findings in human teeth is dangerous from these limited laboratory data, the relationship between these changes and those in periodontosis (or negative bone factor) is suggestive.

Johnson and Bevelander (p. 128) used histochemical technics to study odontogenesis further. The localization of nucleic acid and phosphatase in odontoblasts and ameloblasts suggests that nucleic acids, as well as alkaline phosphatase, have a role in calcification.

Comparative study of chelating agents and other decalcifying agents by Hunter and Nikiforuk (p. 136) gave very favorable results indicating that the chelating agent used was not appreciably affected in its decalcifying ability by temperature, concentration, and pH. This further expands the results discussed by Nikiforuk and Sreebny (J. D. Res. **32**: 859, 1953).

Fosse (p. 139) described an apparatus and technic for ground sections. Any means of improving ground section technic will be a boon to dental histologists and pathologists.

H. B. G. R.

THE EFFECT OF CORTISONE ON THE HEALING OF EXTRACTION WOUNDS IN THE RAT

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A CONSIDERABLE number of clinical and laboratory observations have been reported which indicate that adrenocorticotrophic hormone (ACTH) and cortisone inhibit the growth of granulation tissue and thereby retard the healing of wounds.

Ragan, Grokoest, and Boots[1] were among the first to mention this as an incidental finding in human beings receiving ACTH for the treatment of rheumatoid arthritis. They pointed out that various wounds (biopsy sites, decubitus ulcers and so forth) in these patients were extremely slow to heal. This was subsequently confirmed in human beings by Creditor, Bevans, Mundy, and Ragan,[2] among others.

Numerous experimental studies on animals have also been carried out in further investigation of this problem. Ragan, Howes, Plotz, Meyer, and Blunt[3] have reported that cortisone inhibited the growth of granulation tissue in experimentally produced wounds of rabbits' ears, and Howes, Plotz, Blunt, and Ragan[4] have reported similar findings. The latter authors have also shown that rats receiving relatively large doses of cortisone (10 and 20 mg. daily) exhibit a decreased expansile strength of experimentally induced wounds. This has been confirmed by Alrich, Carter, and Lehman.[5]

The apparent mode of action of ACTH and cortisone in retarding wound healing lies in their ability to inhibit the proliferation of new fibroblasts and new endothelial sprouts.[6] Once new fibroblasts arise, they do not appear to be modified, and this has given rise to the hypothesis that the actual cell inhibited may be the prefibroblast. In any event, it appears most likely that there is not a complete suppression of mesenchymal activity, but rather a delay in its reaction.

Additional studies indicate that the skeletal system may also be influenced by ACTH and cortisone. In 1944, Becks, Simpson, Marx, Li, and Evans[7] reported that ACTH in the rat retards chondrogenesis and osteogenesis in the proximal epiphyses of the tibia. Follis,[8] employing cortisone, has found that in the epiphyses of the tibia there is a marked narrowing of the epiphyseal plate and a great thickening of the zone of bone spicules containing cartilaginous cores beneath the epiphysis. This was interpreted as being due to a decrease in normal osteolytic activity in this region rather than an increase in osteoblastic activity.

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In view of the marked effects of cortisone on wound healing and on bone growth, it is only logical that the effect of this compound on the healing of fractures should have been studied. Blunt, Plotz, Lattes, Howes, Meyer, and Ragan[9] have found that experimental fractures in rabbits treated with cortisone showed delayed healing, the chief features being a decreased ingrowth of new blood vessels and poor reconstruction and differentiation of tissue in the fracture site. Similar findings have been reported by Sissons and Hadfield,[10] who stressed that all the histologic processes of bone repair were retarded, periosteal bone formation was absent, and the cartilage callus was of an abnormal type. In contrast to these two studies, Key, Odell, and Taylor[11] have recently reported no delay in healing or alteration in histologic appearance of experimental fractures in rats. They feel that such findings are difficult to explain in the face of previously reported evidence. However, they point out that the rat has been shown to be extremely resistant to cortisone. Nevertheless, since their animals did show a decreased rate of growth, lack of growth of hair, and decrease in the size of adrenals, and since there was even death of several animals apparently due to toxic effects of cortisone, it is difficult to believe that their dosage of cortisone was insufficient. The study reported here was initiated because it was felt that the extraction wound is an excellent place to follow the dynamic process of the formation of granulation tissue similar to that seen in the healing of a wound by "second intention" and the subsequent replacement of this temporary scaffold by osteogenesis under the influence of cortisone therapy. Furthermore, although it is realized that great care must be taken in attempting to translate the results of animal studies into terms of the human being, it was believed that some insight might be gained into the way in which extraction wounds could be expected to react in patients under treatment with ACTH or cortisone. Now that these compounds have become more than laboratory curiosities and are being used in the treatment of a large number of varied conditions, this has become a very definite potential clinical problem to the dentist.

EXPERIMENTAL METHOD

Sixty-five stock female albino rats, approximately 60 days of age and weighing between 130 and 160 grams, were utilized in this study. Under Nembutal anesthesia, the maxillary right first molar of each animal was extracted by means of a small specially designed elevator. Care was exercised to prevent tearing of the mucosa around the tooth and also to prevent fracturing of any of the roots. Occasionally, however, one or two of the roots were fractured, and it was found that these could not be removed. Any animal in which the crown was fractured was discarded.

On the day of the operation, each of the thirty-five experimental animals was injected intramuscularly with 2.5 mg. of cortisone acetate (11-dehydro-17-hydroxy-corticosterone-21-acetate, Cortone*), and daily thereafter with 2.0 mg. of cortisone acetate (approximately 12.5 mg. per kilogram).

*Merck and Company, Inc.

The animals were sacrificed in groups after extraction such that wounds of the following duration could be examined: two, four, five, seven, ten, fourteen, and twenty-one days. In each group, at least five cortisone-treated animals and four control animals were studied. At sacrifice, the heads were removed, skinned, and fixed in 10 per cent formalin for forty-eight hours. They were then sectioned sagitally in the midline, the mandibles disarticulated, and the right side decalcified in 10 per cent trifluoroacetic acid.* After paraffin embedding, the specimens were sectioned semiserially at 8 μ in a mesiodistal plane and stained with hematoxylin-eosin.

RESULTS

Two-day Extraction Wounds.—The sockets of the control animals exhibited a blood clot composed of the usual red blood cells and strands of fibrin with a considerable number of small patent capillaries around the periphery. Numerous fragments of the periodontal membrane were present around the wall of the socket, and there appeared to be considerable fibroblastic proliferation in the areas of these fragments. The surface of the wound was covered by a thick layer of debris, exudate, and necrotic blood cells. Between this surface layer and the blood clot proper was a dense layer of leukocytes.

The sockets of the cortisone-treated animals exhibited a comparable clot of blood cells, chiefly erythrocytes, entrapped in a network of fibrin strands. There appeared to be a proliferation of fibroblasts and endothelial cells on the periphery of the socket comparable to that found in the control animals. The surface layers of necrotic debris and leukocytes also were similar.

No differences could be demonstrated between the two-day extraction wounds of the control and cortisone-treated animals.

Four-day Extraction Wounds.—The sockets of the control animals showed considerable organization of the clot at this time. There had been marked invasion by fibroblasts and capillaries, and the deposition of many young collagenous fibrils. In some instances there were foci of red cells of the original clot remaining. Around the periphery of the wound, adjacent to the bone, fragments of periodontal membrane still persisted. This supporting bone had begun to show remodeling with intense osteoclastic activity around its margins. Occasionally, very early osteoid formation was noted here.

The sockets of the cortisone-treated animals also exhibited vigorous capillary and fibroblastic proliferation with many delicate interlacing connective tissue fibers being laid down. Osteoclastic activity and the formation of new osteoid also appeared comparable to those in the control animals.

At this stage of healing of the extraction wounds, no differences were noted between the experimental and control groups of animals.

Five-day Extraction Wounds.—In the control animals, little difference was noted between the four- and five-day groups. In the latter group, the remnants of the periodontal membrane had almost completely disappeared,

*Minnesota Mining and Manufacturing Company, St. Paul, Minn.

and the general organization of the clot was proceeding rapidly. A heavy layer of debris and leukocytes still persisted over the surface of the wound.

The cortisone animals also showed this rapid generalized organization of the clot, condensation of the young connective tissue fibers into more mature bundles, and the formation of considerable osteoid. The heavy surface layer of necrotic debris and cells was also evident.

There appeared to be no differences in the healing process of five-day wounds in control and experimental animals.

Seven-day Extraction Wounds.—The healing had proceeded to the point in the control animals where numerous rather large capillaries now traversed the entire width of the socket. The epithelium had not yet completely covered the surface of the wound, and small fragments of necrotic bone were seen near its surface, apparently in the process of being sequestrated. Some variation existed between animals in regard to the number of leukocytes persisting in the socket: a few animals showed considerable quantities, others very few. New bony trabeculae of an immature variety were present, especially near the base of the socket.

The wounds of the cortisone-treated animals exhibited similar findings when this stage of healing was compared to that of the control animals. The process appeared equally well advanced in both groups, there being no evidence of a lag in any particular phase.

Ten-day Extraction Wounds.—The control animals exhibited nearly complete epithelialization of the wounds by this stage. An occasional animal had a small defect of the epithelium, most commonly in areas where small fragments of bone were still being sequestrated. The connective tissue of the socket was now of a more mature variety although still highly cellular. Fibrillar bone was visible, filling in the lower portion of the socket as well as some growth inward from the lateral walls. Osteoblastic activity around the new spicules of bone was particularly vigorous. Occasionally there was a small amount of residual inflammation beneath the epithelium, but this was not a common finding. (Fig. 1, *a*.)

In the experimental animals, complete re-epithelialization had not occurred. Instead, over the surface there was still a thick layer of debris and leukocytes. One common feature noted was that the socket had not been as completely filled in with connective tissue as in the control animals and, therefore, there was a considerable depression of the surface of the wound. The granulation tissue in the superficial portion of the socket was of a younger, more cellular and more vascular variety than that in the control animals. In the base of the socket, however, new bone formation was proceeding at a rate indistinguishable from that in the controls. (Fig. 1, *b*.)

For the first time since the healing process had begun, there appeared to be a difference between cortisone-treated and untreated animals. There was lack of re-epithelialization, persistence of immature granulation tissue in the

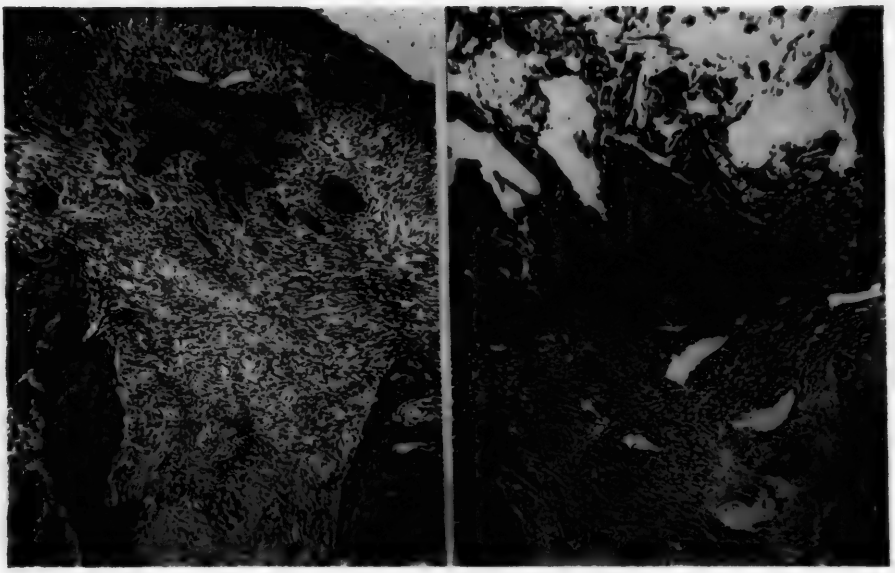

Fig. 1.—*a*, Upper portion of ten-day extraction wound socket of control animal showing complete re-epithelialization, maturation of connective tissue, and relative absence of inflammation; *b*, similar site of ten-day animal which received cortisone showing lack of re-epithelialization and marked inflammation beneath the surface layer of debris and exudate.

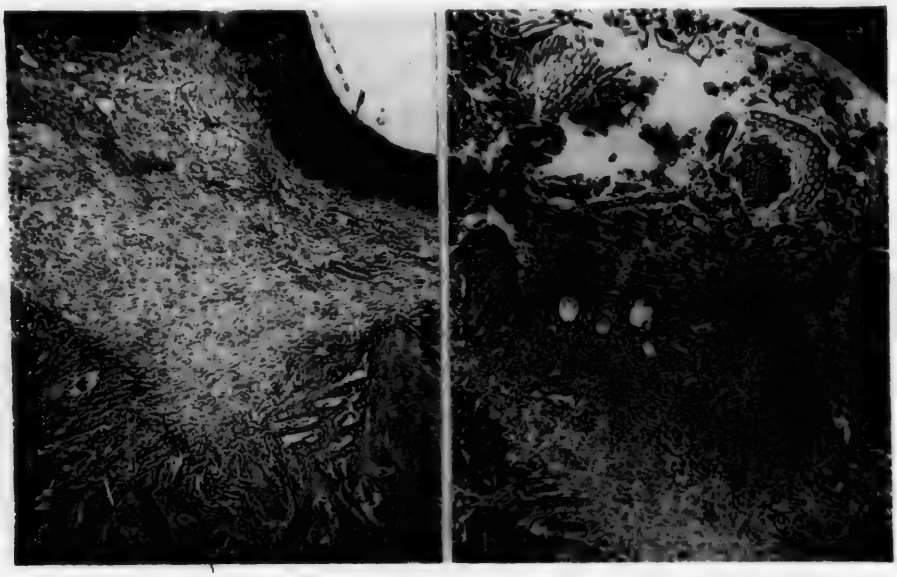

Fig. 2.—*a*, Upper portion of fourteen-day extraction wound socket of control animal showing further maturation of site; *b*, re-epithelialization is still lacking and considerable inflammation is present with surface exudate and debris.

superficial portion of the socket, and a lack of complete re-establishment of height of the socket. The base of the socket appeared to be healing equally well in both groups.

Fourteen-day Extraction Wounds.—A considerable portion of the socket in the control animals had now been filled in with coarse trabeculae of irregular bone. The connective tissue overlying the socket had become arranged into bundles parallel with the surface and had become considerably less cellular. Remodeling of the bony crests was still occurring, as is evident in the osteoclastic resorption occurring here. (Fig. 2, *a*.)

Epithelialization was still incomplete in the cortisone-treated animals, and in the areas of these defects there was a great deal of residual inflammation. Deposition of bone in the socket now lagged considerably behind that of the control animals, and the osteoblasts were less prominent around the trabeculae in the cortisone animals. The connective tissue higher in the sockets was of a more immature variety, and a retardation of the healing process was now evident. (Fig. 2, *b*.)

Twenty-one Day Extraction Wounds.—In the control animals, it was found that the sockets had been almost completely filled with bone in most cases although, due to considerable remodeling of the alveolar crests, the original height had not been restored. This new bone was coarse, and exhibited little interstitial tissue. There was a small amount of residual inflammation in the subepithelial connective tissue in one animal, but re-epithelialization was complete.

The sockets of the experimental animals exhibited little difference from those of the control animals. The extent of bony replacement was approximately equal in both groups. However, several of the cortisone animals still exhibited incomplete epithelialization of the wound, incomplete sequestration of small bony fragments, and some residual inflammation. Thus, while healing of the socket compared favorably with that in the control animals, healing of the superficial soft tissue still appeared somewhat delayed.

DISCUSSION

In view of the reports in the literature describing retardation of the healing of both soft tissue wounds and fractures of bone, in animals and human beings treated with ACTH and cortisone, it is somewhat surprising that this was not a more prominent feature in the healing of extraction wounds in cortisone-treated rats. As Key, Odell, and Taylor[11] have suggested, however, and as many of the experimental studies indicate, the dosage of these compounds is an extremely important factor. Marked variation has been shown to exist between species in their reactions to a specific dose of cortisone, and the rat appears to be one of the most resistant of the common laboratory animals.

To illustrate, in the studies of Ragan, Howes, Plotz, Meyer, and Blunt[3] on rabbits it was shown that 25 mg. of cortisone daily (approximately 8 to 10

mg. per kilogram) markedly delayed the appearance of granulation tissue in experimental wounds of the ear. Similar dosage in rabbits delayed the healing of fractures in the studies of Blunt, Plotz, Lattes, Howes, Meyer, and Ragan[9] and Sissons and Hadfield.[10] In the rat, Howes, Plotz, Blunt, and Ragan[4] have shown no significant difference in expansile strength of stomach wounds between control animals and animals receiving 20 mg. per kilogram. A difference was found at a level of 40 mg. per kilogram. Spain (quoted by Ragan[6]) found that granulation tissue in mice was inhibited by a dose equivalent to 100 mg. per kilogram. No effect on wound healing was noted by Ragan[6] in the dog given 7.5 mg. of cortisone per kilogram. In the fracture healing studies by Key, Odell, and Taylor,[11] rats receiving approximately 10 to 20 mg. of cortisone per kilogram showed no delay in the repair process. These animals did exhibit a decreased rate of growth, lack of hair regrowth, and decrease in the size of the adrenals. Thus, the cortisone appeared to produce some systemic effects although not specifically on the healing phenomenon.

In the study reported here, the only physical evidence that the animals had responded to cortisone was the fact that all treated animals showed a marked progressive loss of weight such that in the fourteen and twenty-one day groups, there was considerable doubt that the animals would even survive the experimental period.

The criticism may be applied that if a state of hyperadrenalism had been induced before the extractions were carried out, a delay in healing may have been shown in the earlier wounds. This possibility remains for future investigation.

It is to be pointed out, however, that there was some histologic evidence of delayed healing in the ten- and fourteen-day treated animals and possibly in the twenty-one day animals. The chief defect did not appear in the new bone formation in the sockets but rather as a retardation of maturation of the connective tissue replacement, slow re-epithelialization of the wound, and a prolonging of the sequestration process and its associated inflammatory reaction.

In general, the healing of the extraction wounds in the control animals was comparable to that reported in the Long-Evans strain of rat by Huebsch, Coleman, Frandsen, and Becks.[12]

SUMMARY

1. Study of healing of extraction wounds in cortisone treated rats showed no evidence of retardation of the healing process until the tenth day, when lack of maturation of granulation tissue and lack of re-epithelialization was noted. By the twenty-first day, incomplete epithelialization was still prominent, as well as incomplete sequestration and residual inflammation.

2. There appeared to be little interference with the formation of new bone in the sockets throughout the experiment in the treated animals.

3. The findings are not in complete accord with other wound healing studies in different species and may be partially explained by the relative resistance of the rat to cortisone.

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A STUDY OF AMALGAM CONDENSATION PROCEDURES WITH EMPHASIS ON THE RESIDUAL MERCURY CONTENT OF THE INCREMENTS

I. Strength, Flow, and Dimensional Change

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ALTHOUGH amalgam still remains the most commonly used restorative material and a vast amount of laboratory and clinical research concerned with its use has been published, there are still many practical problems that remain unanswered. One of these is the consistency, or actually the mercury content, of each increment which is inserted into the cavity preparation during the condensation procedure. It is generally recognized that the residual mercury content of the restoration will influence tarnish and strength. However, the dryness of the individual increments which are used to build the restoration remains a controversial issue.

The most common and oldest method is one which employs a somewhat wet initial portion, followed by increments of increasing dryness, and culminated by a final dry portion wrung in a squeeze or chamois cloth.[1-3] The subsequently drier portions supposedly act as a blotter to absorb the excess mercury as it is worked to the top of the restoration. Advocates of this procedure feel that initial plastic portions provide better adaptability and that with proper condensation pressures and technics, the over-all residual mercury content is at a minimum.

However, in recent years some outstanding amalgam operators have recommended the use of relatively dry increments throughout the restoration.[4-6] Advocates of this technic believe that a more homogeneous filling with resulting better physical properties is produced in this manner. One of the arguments presented for this technic is that the mercury content will be approximately the same throughout the restoration and thus the physical properties will be constant.[7] The technic of using a uniformly dry mass of amalgam has increased in popularity since the advent of mechanical condensers, although the "increasing dryness" technic previously mentioned is undoubtedly still more widely used. No research has been reported on this

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very practical phase of amalgam manipulation. Thus this investigation was designed to determine which technic would consistently produce better physical properties.

PROCEDURE

Four widely used condensation procedures, variations of the two basic ones described, were employed in this study.

(a) This is the so-called increasing dryness method in which a moderate amount of mercury was expressed from the first increment by finger pressure. Slightly more mercury was expressed from each of the succeeding increments, and the final portion was wrung dry in a squeeze cloth. It should be emphasized that the first increments which were inserted were not extremely wet or sloppy. A sufficient amount of mercury had been removed to provide a mass that would offer resistance to the condensing instrument; thus the amalgam was not just flowed or puddled into the mold. There was no attempt in this procedure, or in any of the others, to utilize any method, such as special pliers, for expressing a given amount of mercury. Such devices are not routinely used, and the primary concern of this investigation was to compare the procedures as they are actually utilized in the dental office. The investigators so perfected their technics that with each method individual specimens could be prepared with consistent results.

(b) A squeeze cloth was used to remove approximately equal amounts of mercury from each segment. No mercury was removed from the mix until each segment was prepared for condensing. The consistency in these increments was comparable to the middle portions used in Technic a.

(c) The mercury was expressed from the entire mass with a squeeze cloth immediately after mixing. During condensation the remaining portion of the mix was continually being softened by an assistant, who was mulling it in a rubber dam. If possible, additional mercury was removed by the squeeze cloth from each increment of the amalgam before insertion.

(d) The mercury was expressed from the entire mass immediately after mixing, as in Technic c. However, the amalgam was not mulled by an assistant during condensation nor was any additional mercury expressed from individual increments before carrying to the mold.

In all these technics, never was the amalgam squeezed so dry that mercury could not be worked to the surface during the packing procedure. Sweeney[4] and Miller[5] have cautioned against the laminating effect and surface roughness produced from use of such a consistency. Thus, never, even with the amalgam used for the final overpacking, was the maximum amount of mercury expressed. The investigators feel that this is an important factor to be considered in evaluating these technics.

Both hand and mechanical condensation were used with these four methods of packing. These two types of condensation were employed in order to discern whether any one of these methods might be more adaptable to mechanical condensation. A heavy hand pressure (approximately seven pounds), an impact type of condenser, the Mid-West, and a vibratory condenser, Kerr, were utilized. The condensing point of the hand instrument was 2 mm. in diameter, while a 3.5 mm. condenser point, of the type recommended by Sweeney,[4] was used with the mechanical condensers. No attempt

was made to standardize the size of the condenser point since this study was not primarily concerned with a comparison of hand versus mechanical condensation. A small-grained alloy,* same batch, was used throughout. The alloy:mercury ratio was carefully controlled at 5:8 by means of a Crandall balance. In order to insure uniformity of mixes, the amalgam was triturated in a Crescent Wig-L-Bug. Wherever the technic required the use of a squeeze cloth, a Kerr nylon cloth was employed. Approximately six minutes was required for condensing the dimensional change and compressive strength specimens, and four minutes for the flow specimens. Very small increments were used to fill the mold.

Physical property tests were run in accordance with American Dental Association Specification 1. Flow, dimensional change, one hour and twenty-four hour compressive strengths were measured. The rate at which the load was applied to the compressive strength specimen was carefully controlled,[8] all specimens being loaded at the rate of 15,000 pounds per square inch per minute. The flow specimens were also used for measuring the residual mercury content. The specimens were first pulverized by grinding with a very slow-moving diamond bur, and were then chemically analyzed for mercury by the method described by Schoonover and Caul.[9] All analyses were run in triplicate, and the average results are reported.

Two different operators prepared specimens for each technic. It was found that the results obtained by the two investigators compared favorably, and thus the following data are a composite of all tests. Compressive strength figures represent an average of ten individual specimens while a minimum of six dimensional change and flow specimens were made for each packing procedure. A total of 385 specimens were made in measuring the physical properties, and 47 specimens have been analyzed for mercury content.

RESULTS

Examination of the one-hour strength data summarized in Fig. 1 does not indicate that any of the condensation technics greatly affected the one-hour compressive strength of amalgam. It may be noted that the impact type of mechanical condensing seemed to have a tendency to increase very slightly the one-hour strength with each technic. Phillips[10] and Ryge[11] have shown this in other studies. The difference might have been somewhat higher if the size of the condenser point had been the same. The only technic to give a consistently but slightly higher early strength, with all three condensers, was Method d. It will be recalled that in this procedure all the mercury was expressed immediately from the entire mix with no mulling of the remaining amalgam. It would be expected that such a technic might give somewhat higher one-hour data since the amalgam would set more rapidly. However, in the twenty-four hour strengths (Fig. 2) this technic did not give a higher ultimate strength. Again it can be seen that there is no significant difference in any of the four methods, either by hand or mechanical condensation.

*Baker, Aristaloy.

No apparent relationship exists between the condensation technic and the flow (Fig. 3). Technic a, the increasing dryness method, was superior in hand packing while Technic b was best with the mechanical condensers. The maximum difference, however, with all four procedures was less than 1 per cent, and the flow in all cases was within the specification limit.

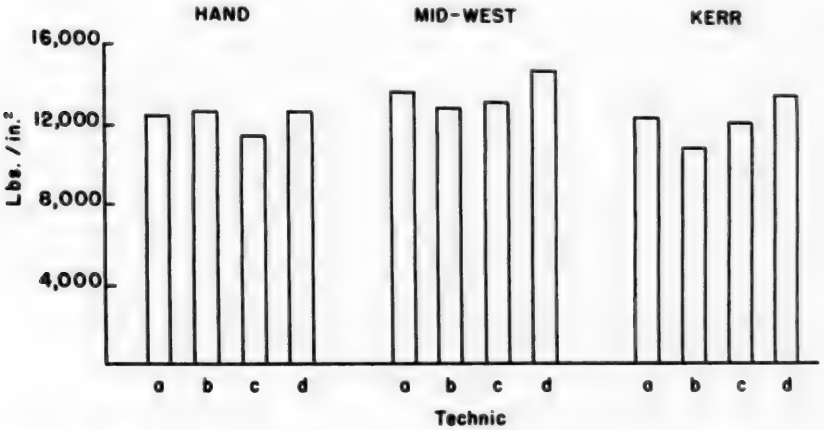

Fig. 1.—Effects of various condensation technics on one-hour compressive strength of amalgam.

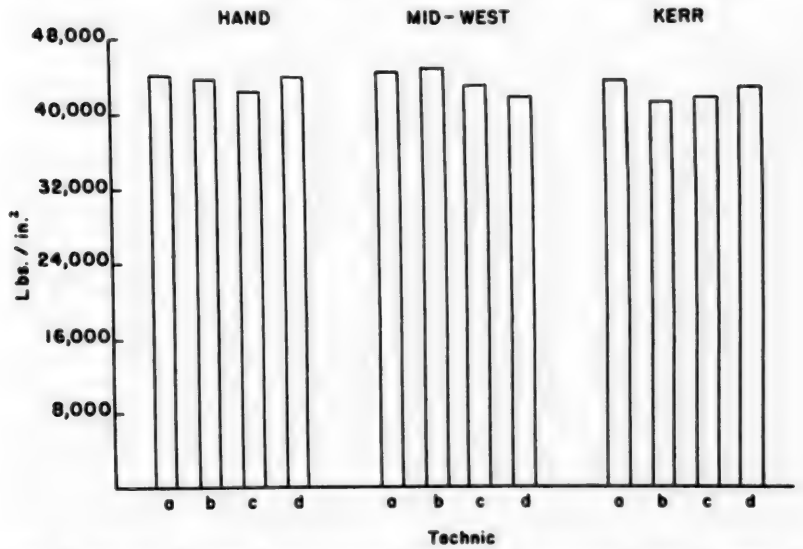

Fig. 2.—Effects of various condensation technics on twenty-four hour strength of amalgam.

The results on dimensional change are shown in Fig. 4. All technics produced some contraction. This would be expected since thorough trituration and use of the mechanical amalgamator are known to cause contraction with

most alloys.[10, 12] There is no evidence to indicate that dimensional change of this order is clinically significant.[13, 14] Technic c, both with hand and mechanical condensation, did produce the least change.

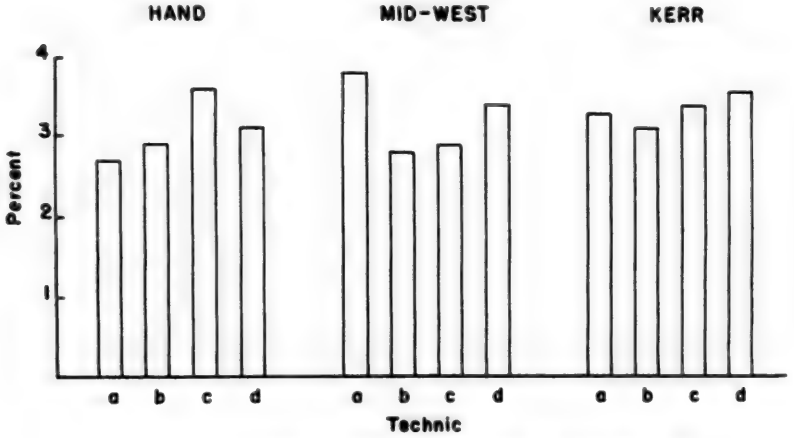

Fig. 3.—Effects of various condensation technics on flow.

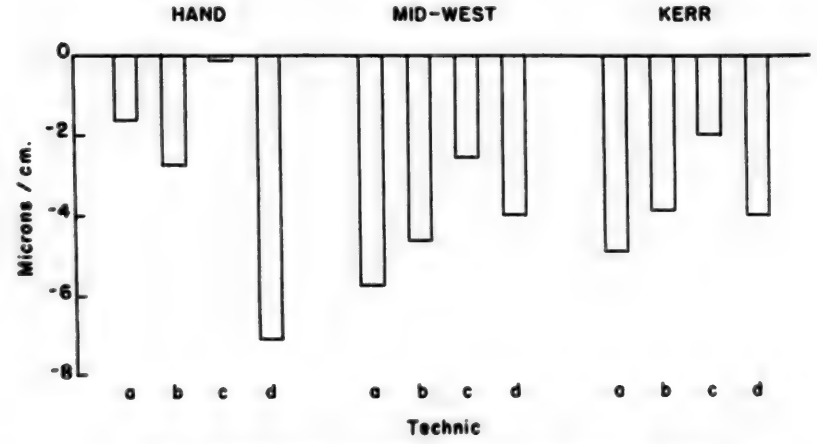

Fig. 4.—Dimensional changes produced by various technics.

Only small differences in residual mercury content were noted in specimens packed in various ways (Fig. 5). Even though the mercury content of those packed by mechanical condensing appears to be very slightly less than in those packed by hand, these differences are not considered significant, particularly since examination of individual data revealed that the residual mercury content of specimens packed in exactly the same manner varied in some cases as much as 2 per cent.

No correlation was demonstrated between mercury content of the entire specimen and flow. This corroborates the findings of Ryge, Dickson, Smith, and Schoonover,[11] who could show no relationship between physical properties and mercury content with various types of condensing instruments.

Although these data do not show any significant difference in the residual mercury present in the entire specimen when the four methods were employed, additional tests are being made to determine the mercury content of the individual increments both before and after condensation. It is felt that such data might be more valuable in determining exactly how effective each procedure is in eliminating the excess mercury, and which method will give a more homogeneous restoration.

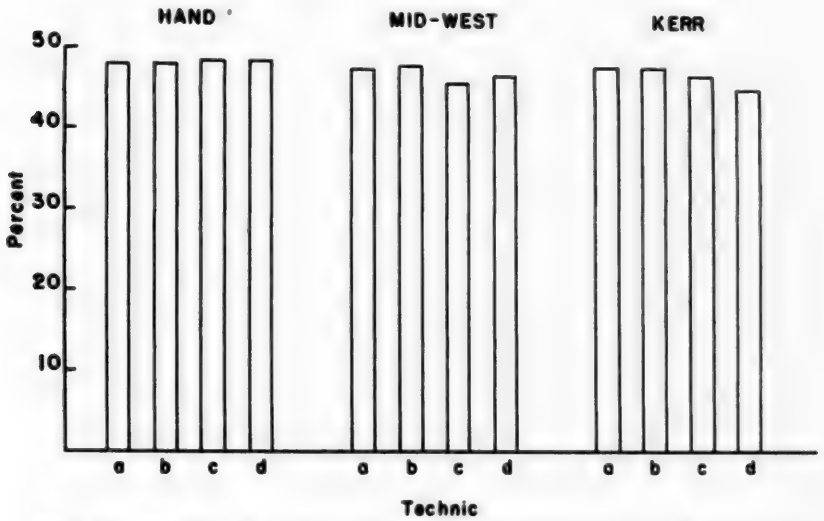

Fig. 5.—Differences in mercury content among specimens packed in various ways.

The data were analyzed statistically in order to determine whether more consistent results were obtained on individual specimens by one technic than by another. Since it would be expected that the greatest variation might occur in the one-hour compression test, the standard deviation of these data was calculated. It can be seen in Table I. No one technic seemed to produce results of much greater consistency than another except for Technic b, when the mechanical condensers were employed. The data again compared favorably with those of Ryge and associates,[11] who, although they did not compute the standard deviation, stated that in their study the variations in individual determinations for three compressive strength specimens were approximately 1,000 pounds per square inch. It is of interest to note that the hand procedures actually gave somewhat more consistent results than the mechanical devices here used.

TABLE I. STANDARD DEVIATION

TECHNIC	INSTRUMENT		
	HAND	MID-WEST	KERR
a	730.6	1,863.0	1,629.7
b	842.4	1,000.6	837.4
c	619.5	1,852.1	997.3
d	730.9	1,685.4	1,659.7

There have been no attempts in this paper to evaluate these procedures from the standpoint of ease of manipulation or insertion. Technics c and d, of course, require more speed on the part of the operator and are better adapted to mechanical condensation than to hand condensation.

SUMMARY

Amalgam was condensed employing four different procedures for the removal of mercury from the individual increments during its condensation. In none of the procedures, however, were increments used where the mercury content was either insufficient or too great for practical use. Both hand and mechanical condensing were employed. No correlation could be noted between the physical properties or residual mercury content and the specific method of packing.

On the basis of work completed thus far it would seem that if a fundamentally sound technic for removing mercury from the individual increments is accompanied by a carefully standardized condensation procedure it makes little difference in the physical properties which technic is employed for removing the residual mercury. However, this must be regarded as a progress report since many factors remain to be studied before definite conclusions can be reached.

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ELECTRON MICROSCOPY OF CARIOUS DENTIN

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INTRODUCTION

HISTOLOGIC studies of the carious lesion in dentin customarily differentiate it into four zones of degeneration based on the degree of bacterial invasion. The first region, the most superficial, is characterized by complete decalcification and decomposition of the dentinal tubules and matrix. Below this zone, the dentin shows incipient decalcification. Underneath this area, the tubules have become obliterated by the calcification of the dentinal fibers, the so-called zone of transparency. The fourth and innermost layer consists of fatty degeneration of the dentinal fibers with beginning calcification. Zones one and two are caused by microbial action while the other two regions are the result of the metabolic disturbance affecting the dentinal fibers which are reacting to irritation.

This separation into definite regions is based on optical microscopic studies, using ground and decalcified sections. However, with the development of ultrathin sectioning methods for the direct observation of hard tissues under the electron microscope, a new medium has been provided for the study of the different layers of carious dentin.

The present report describes the minute microscopic changes occurring in carious dentin as observed in thin sections with the electron microscope. These alterations are not revealed by methods previously employed.

MATERIALS AND METHODS

In this study, the carious teeth were collected from patients attending the Surgery Clinic of the School of Dentistry, University of Southern California. Immediately after extraction the roots of the teeth were clipped off, enabling faster penetration of the fixative through the pulp. The fixatives used were 1 per cent osmic acid and Bouin's fixative. Bouin's fixative was found to give the best results for the cytoplasmic inclusions of the microorganisms. The teeth were kept in the fixative for at least twenty-four hours.

The specimens were decalcified in 10 per cent nitric acid in formalin, and prepared for sectioning by the paraffin nitrocellulose method. The teeth were embedded in 40 per cent nitrocellulose which was hardened in chloroform, and then infiltrated with 65° C. paraffin. The blocks were kept about 1 mm. square. Preliminary to electron microscopy, sample sections of 1 micron were

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always observed under the optical microscope to determine the orientation of the sections with respect to the degree of caries penetration. These sections were stained with hematoxylin and triosin and Gram's stain for the demonstration of bacteria. For electron microscopy, sections of 0.05 micra were obtained using the modified microtome described by Pease and Baker.[10] Micrographs were then taken of the desired areas.

OBSERVATIONS

The most superficial zone of carious dentin is described as being composed of a soft crumbly mass. Fig. 1 is an electron micrograph of such an area. There is no resemblance to normal dentin architecture. The mass consists of a heavy concentration of microorganisms of the coccoid and bacillary types. One cannot determine with certainty which variety predominates since many of the rods are cut obliquely or in cross section, thus appearing coccoid in form. The bacteria appear to be digesting the remains of the dentinal matrix and the fine branches of the dentinal fibers. Below this region the dentin is undergoing partial decomposition, as shown by the absence of dentinal tubules and the fragmentation of the matrix (Fig. 2). These fragments are being attacked by the surrounding microorganisms. The mode of dissolution is of interest as it appears lacunar in nature, similar to that seen in other hard tissues as the result of osteoclastic activity. An abundance of bacteria is seen in the area. The bacilli may show characteristic pleomorphism, which is best seen in a longitudinal section (Fig. 3). These microorganisms appear ovoid, diplococcoid, and filamentous in shape. The filamentous forms show many vacuoles and inclusion bodies in their cytoplasm.

Beneath this zone of complete dissolution, the dentin is undergoing incipient decalcification and proteolysis as a result of the action of the invading bacteria in the tubules (Fig. 4). Here it can be seen that the normal tubular content is completely destroyed. As a result of this destructive action, and bacterial growth, the shapes of the tubules are changed from ovoid to polygonal, resulting in a decrease in the amount of the intertubular matrix. The dentinal fiber or the protoplasmic extension of the odontoblast has become necrotic, and the lumen of the tubule is occluded by the bacteria (Fig. 5). Intracellular vacuoles are present in a few of the bacteria, and their significance is undetermined at present. As a result of the products of the bacteria acting on the matrix, there is now an indication of the presence of the collagen fibrils of 640 A striations, which in normal dentin are masked by the unaltered ground substance and minerals.

The processes of intratubular bacterial growth and the gradual dissolution of the matrix continue until the adjacent tubular walls are finally disrupted (Fig. 6). The microorganisms migrate into the remaining matrix, and by their action this framework is transformed into a diffuse mass, as shown in Figs. 1 and 2. When this break-through occurs, the collagen fibrils are exposed to the enzymatic action of the bacteria, resulting in their disappearance.

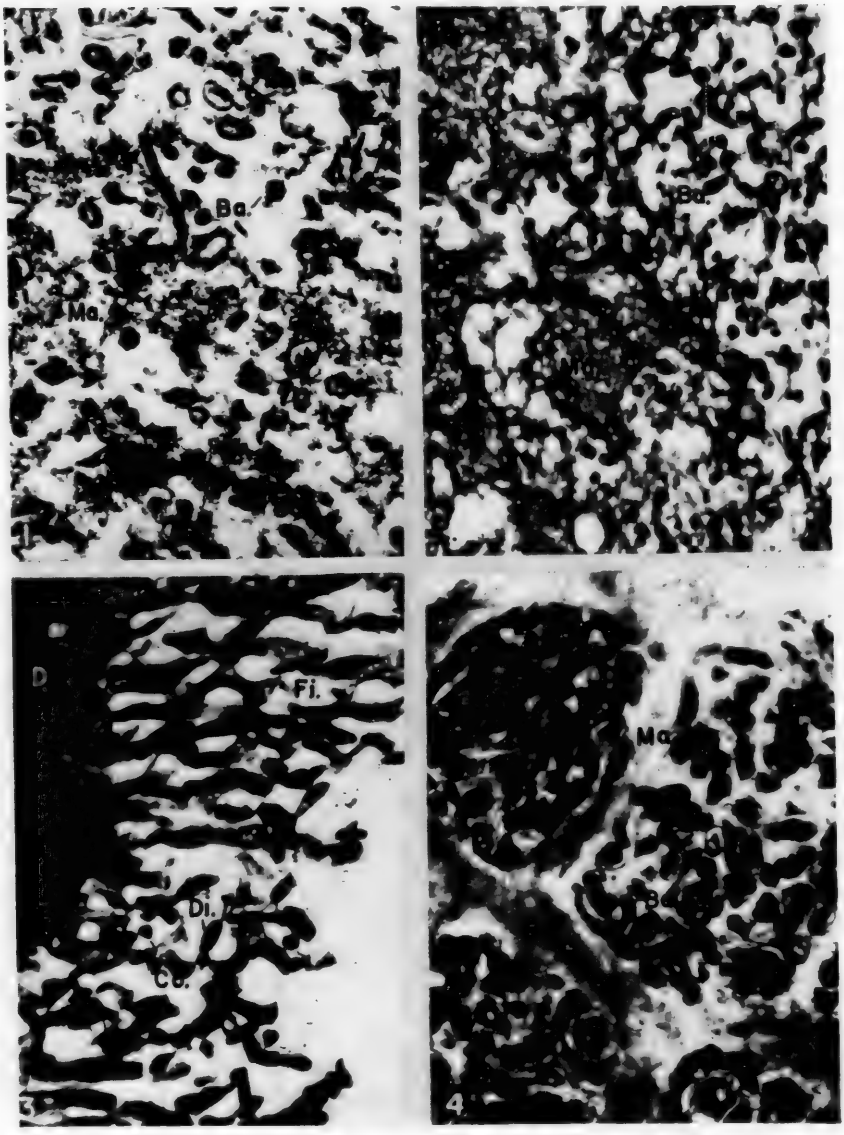

Fig. 1.—Zone of complete decalcification and decomposition showing remains of the matrix and masses of bacteria. *Ba.*, Bacteria; *Ma.*, dentinal matrix. Original magnification, ×9,600; reduced ½.

Fig. 2.—Zone of partial decalcification. Note the lacunar nature of the bacterial dissolution of the matrix. *Ba.*, Bacteria; *Ma.*, dentinal matrix. Original magnification, ×9,600; reduced ½.

Fig. 3.—Microorganisms on the surface of carious dentin showing pleomorphism. *D*, Dentin; *Co.*, coccuslike; *Di.*, diplococcoids; *Fi.*, filamentous forms. Original magnification, ×20,000; reduced ½.

Fig. 4.—Zone of incipient decalcification. Note the bacterial invasion and distention of the tubues. *I.T.*, Infected tubule; *Ba.*, bacteria; *Ma.*, matrix. Original magnification, ×18,000; reduced ½.

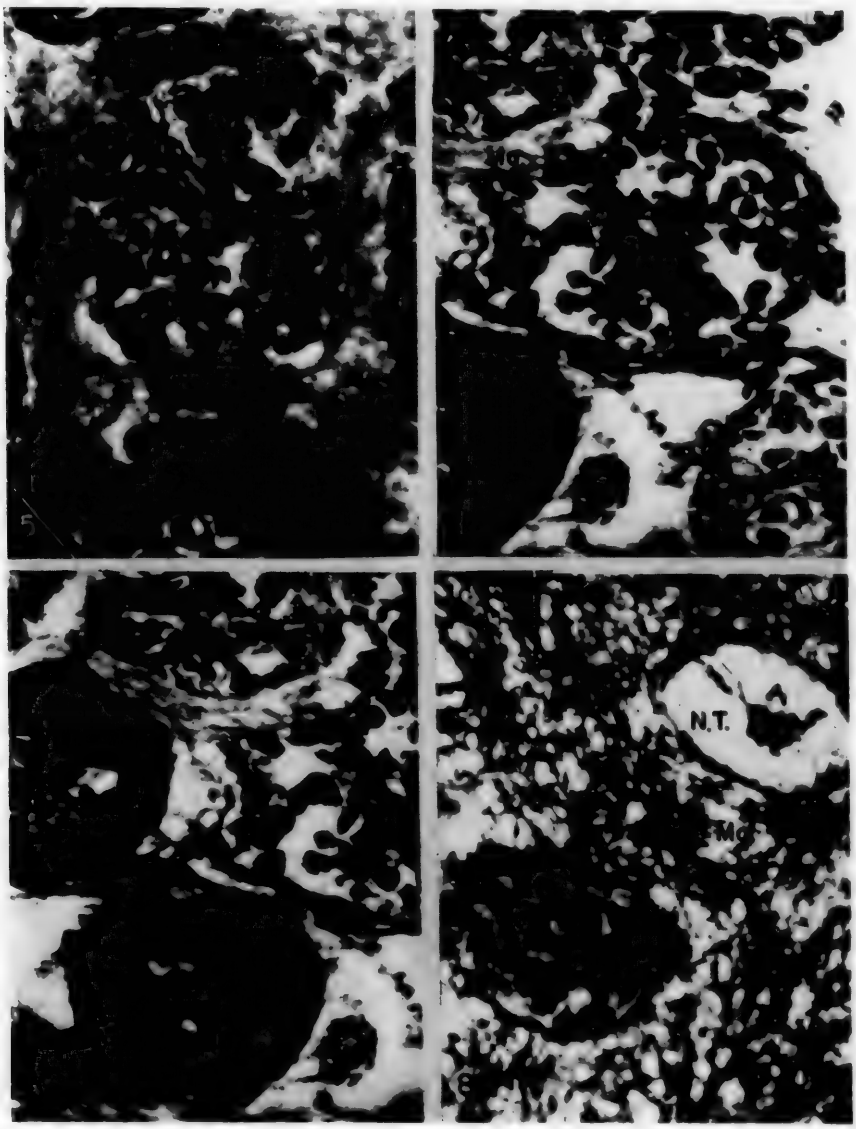

Fig. 5.—Zone of incipient decalcification. Note the polygonal shape of infected tubule and sclerosed tubule below. *I.T.*, Infected tubule; *S.T.*, sclerosed tubule; *Ma.*, matrix. Original magnification, ×22,000; reduced ½.

Fig. 6.—Zone of incipient decalcification. Note the breakdown of tubular wall with migration of bacteria into surrounding matrix. *Ba.*, bacteria; *Ma.*, matrix; *S.T.*, sclerosed tubule. Original magnification, ×22,000; reduced ½.

Fig. 7.—Zone of incipient decalcification showing two sclerosed tubules. *S.T.*, Sclerosed tubules. Original magnification, ×22,000; reduced ½.

Fig. 8.—An area at a level far ahead of actual decalcification showing an infected and normal tubule. *N.T.*, Normal tubule; *I.T.*, infected tubule; *Ma.*, dentinal matrix. Original magnification, ×22,000; reduced ½.

In the process of caries penetration through the dentin, a number of tubules may undergo regressive changes due to the products secreted by the bacteria. In Fig. 5 the content of the tubule in the lower portion of the section is now homogenous in nature, and completely occludes the lumen. This material appears similar to hyaline. In the center of this hyalinized mass, bacteria are present and reacting on this occlusion. In contrast to the surrounding tubules, the number of bacteria present internally is much smaller, indicating that these sclerosed tubules do act as a defense mechanism against the migration of the bacteria through the tubules. The degenerative process may continue with the tubular content becoming calcified, as illustrated in Fig. 7. As in Fig. 6, the number of bacteria in these calcified tubules is not as great as in the surrounding area.

Deep in the dentin, at a level far ahead of actual dissolution, there may be a few tubules that are invaded by the microorganisms. An electron micrograph of such a region is shown in Fig. 8. In this section two tubules are visible. In the normal tubule (*N.T.*) the dentinal fiber appears as a condensed mass in the core of the tubule. This condensation is due to the inability of maintaining proper relationship because of contemporary fixation and decalcification methods. There is also a condensation of the tubular wall. This is an artifact and not the so-called sheath of Neumann, this fact being demonstrated by Bernick, Baker, Rutherford, and Warren[2] in electron micrographs of normal dentin. The other tubule (*I.T.*) is filled with bacteria, and its dentinal fiber is destroyed. The tubular shape is still ovoid, and its diameter remains within normal range. The structure of the intertubular matrix is unchanged.

DISCUSSION

With the application of the electron microscope in the study of the histopathology of caries in the dentin, it becomes desirable to discuss a few controversial observations which exist in the literature.

It has been demonstrated by Engel,[5] using histochemical methods, that in carious dentin the glycoprotein of the ground substance becomes depolymerized. This depolymerization is of bacterial origin, the acid produced by bacteria bringing about initial alteration of the ground substance. This allows the proteolytic enzymes, especially collagenase, to depolymerize the carbohydrate protein complex, resulting in swelling and the dissolving of the ground substance. This thesis is supported by the electron micrographs of the carious lesion. In normal untreated dentin, the matrix is made up of ground substance, collagen fibrils of 640 A striations, and minerals. The fibrils are masked by the other two constituents and do not show in the micrographs of normal dentin. On the other hand, these fibrils become visible in caries, especially in the zone of incipient decalcification where the bacterial enzymes are acting on the surrounding matrix even though the bacteria are still in the tubules. These fibrillar striations disappear when the microorganisms break through the tubules, and by their selectivity act on the col-

lagen elements. When a deeper layer of dentin is viewed, particularly at a level far ahead of decalcification, the fibrils remain concealed, as the ground substance is probably unaffected at this time.

The morphology of the microorganisms in the tubules has been known for over one-half century, ever since Miller[8] described three types of bacteria, cocci, bacilli, and filaments. Each of these supposedly has a tendency to grow individually in separate tubules, although all three types of bacteria are found intermixed in the zone of complete decalcification and dissolution. Tunnicliff and Hammond[12] proposed that these various organisms are just different forms of streptococci. To determine with a degree of accuracy the specific types of bacteria which invade the tubules is very difficult, especially with the use of decalcified sections. The explanation for this situation is found in the arbitrary plane of sectioning in which many bacilli, filaments, and even cocci may be cut in different planes; thus a bacillus or filament cut in cross section would appear spheroid, and would give the impression that an individual tubule may contain only cocci.

There has been considerable discussion concerning the nature of transparent tubules. Many observers (Thoma,[11] Miller,[9] Beust[4]) considered this a degeneration as the result of a vital reaction on the part of the dentinal fibers in which they become calcified. Weber,[13] Euler and Meyer,[6] and others believed that this process first starts as fatty degeneration of the fibers, and calcification of the degenerated structures followed in a manner similar to that occurring in blood vessels.

In the material studied, the sclerosed tubules were found in the zone of incipient decalcification around infected tubules. These sclerosed tubules were undergoing degeneration with the transformation of the protoplasmic extensions of the odontoblasts into a homogenous mass similar to hyaline. This type of regression is probably a response to the products of the bacteria, and is no different than degeneration exhibited by connective tissue found in other parts of the body. It is well known that degenerating and necrotic tissues have an affinity for calcium, and it is no wonder, therefore, that many of these tubules become calcified in the process of caries invasion. These degenerated tubules have a functional significance in that they hinder the rate of migration of the bacteria through the dentin, for the number of bacteria within these sclerosed tubules was always less than in the surrounding tubules.

SUMMARY

Detailed histologic studies of carious dentin with the electron microscope have been undertaken by the use of ultrathin sections. Electron micrographs are presented which demonstrate the different zones of degeneration based on degree of bacterial invasion. The most superficial zone is characterized by complete decalcification and decomposition of the dentin. An area of incipient decalcification in which the bacteria have invaded the tubules is the second zone. In this region, sclerosed tubules are found with their lumina obliterated by either a homogenous or a calcified material. Collagen fibrils of 640 A stri-

ations are also evident in this region. In the deepest layer from the area of decalcification, a few tubules contain microorganisms.

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THE EFFECT OF A TYPICAL INFANT'S DIET ON THE CARIES INCIDENCE OF THE SYRIAN HAMSTER

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MANY common infant formulas have compositions generally similar to those which have been found by numerous investigators to cause an extensive development of dental caries in rats and hamsters. The usual infant formulas consist primarily of a type of commercially processed carbohydrate and of milk in some form, with various types of vitamin supplements. Schweigert, Shaw, Phillips, and Elvehjem[7] have shown that cotton rats on diets generally of this type and containing 73 per cent sucrose, Dextri-Maltose, glucose, or fructose have a high incidence of dental caries. Shafer[8] has found that a diet containing 61 per cent sucrose in the form of powdered confectioner's sugar results in the highest caries score when compared with starch and glucose under similar conditions. Mitchell and Shafer[4] have reported for hamsters that a diet containing 45 per cent sucrose is associated with slightly more caries and lower terminal weights than 30 and 15 per cent amounts of sucrose.

Because of the similarities of infant formulas to diets which are cariogenic for animals, and because of the possible importance of this factor in the choice of formulas by pediatricians, a preliminary study of the cariogenicity of one type of such diets was carried out and is described in the present work. A limited study of the effect of the inclusion of sodium citrate in a cariogenic diet was also studied because of the observation by Carlson, Farina, and Whiteside-Carlson[1] that citrates may inhibit the growth of *Lactobacillus acidophilus*.

Two diets generally accepted as being cariogenic for hamsters were also studied in a series of three experiments to obtain information concerning their relative cariogenicity and reproducibility. The Syrian hamster (*Cricetus auratus*) was used as the experimental animal in this work. The carious process in this animal more closely resembles that found in human subjects than does the carious process occurring in the rat. This advantage, however, must be balanced against the lack of knowledge of nutritional factors for the hamster as compared with the rat.

EXPERIMENTAL

The experimental animals were obtained from an inbred hamster colony maintained on Purina Laboratory Chow with a weekly lettuce supplement. All animals were weaned at 17 days of age, distributed by sex and litter mate

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as evenly as possible among the experimental diets, and kept on the chosen diet with a weekly supplement of lettuce for fifty-eight days. Tap water was available at all times. The complete set of experiments was repeated over three successive periods. At the end of fifty-eight days, the animals were sacrificed and the heads placed in a colony of dermestid beetles to be cleaned. After being cleaned in this manner, the heads were soaked overnight in 2 per cent ammonium hydroxide and allowed to dry. Molar teeth were examined with a microscope at thirteen- and thirtyfold magnification. The carious lesions were recorded and scored according to the method of Keyes[2] except that stained areas were not scored unless there was definite loss or softness of the calcified tissue.

The experimental diets consisted of the following:

Diet A: Purina Laboratory Chow with weekly supplement of fresh lettuce.

Diet C: 22 per cent granulated sucrose, 44 per cent Pearl-grade corn starch, 30 per cent powdered whole milk, 3 per cent powdered alfalfa, and 1 per cent sodium chloride.

Diet D: 22 per cent granulated sucrose, 39 per cent Pearl-grade corn starch, 5 per cent sodium citrate, 30 per cent powdered whole milk, 3 per cent powdered alfalfa, and 1 per cent sodium chloride.

Diet E: 66 per cent granulated sucrose, 30 per cent powdered whole milk, 3 per cent powdered alfalfa, and 1 per cent sodium chloride.

Diet F: 38 per cent Dextri-Maltose,* 62 per cent powdered whole milk, and 1 c.c. daily of a 1:100 dilution of ABDEC commercial vitamin preparation given orally by means of an eye dropper.

The total water-soluble carbohydrate was determined using the anthrone reaction described by Morris.[5] The diets were extracted three times with boiling water, the combined extracts were diluted to a known volume, and aliquots of the clear extract were taken for analysis. The values recorded in Table I are average values of duplicate determinations. Over the range 0.01 to 0.20 mg. of glucose, a linear relationship between the glucose concentration and the color produced by reaction with the anthrone reagent was established. The intensity of the color was measured with the Klett Photoelectric Colorimeter using a No. 66 (red) filter. Dilutions were made so that the sugar concentrations fell within this range. All values are reported as apparent "glucose."

The fluoride content of the diets was determined by the modification of the Williams[11] titration described by Smith and Gardner[10] after preliminary ashing and distillation from perchloric acid as described in the Official and Tentative Methods of the Association of Official Agricultural Chemists. Duplicate determinations were made, and the average values are recorded in Table I.

The moisture determinations were made by drying weighed samples of the diets to constant weight at 110° C. The average moisture values for duplicate determinations are recorded in Table I.

*A commercial enzyme hydrolyzed starch (Dextri-Maltose) containing 55 per cent reducing sugar as maltose. This contains D-glucose, maltose, and dextrins.

TABLE I. ANALYSES OF EXPERIMENTAL DIETS

DIET	TOTAL WATER-SOLUBLE CARBOHYDRATE* (PER CENT)	MOISTURE (PER CENT)	FLUORIDE* PPM	TERMINAL WEIGHTS OF ANIMALS	
				NO.	WEIGHT (AVERAGE) (GM.)
A	28.2	10.0	30.2	9 ♂	92
				6 ♀	96
C	78.2	6.8	2.3	10 ♂	89
				9 ♀	76
D	70.5	6.2	1.1	4 ♂	79
				3 ♀	75
E	74.0	2.9	1.8	9 ♂	71
				8 ♀	85
F	61.8	6.2	2.8	9 ♂	116
				7 ♀	101
Drinking (Tap) Water			0.03		

*Dry basis.

The average terminal weights of some of the experimental animals are recorded in Table I to serve as a general indication of the nutritional effects of the different diets.

RESULTS

The caries incidence of the hamsters on the various diets is shown in Tables II and III. Analyses are given in Table I for total water-extractable carbohydrate, moisture, and fluoride; the average terminal weights of some of the experimental animals are indicated in Table I. The standard deviation of the mean score for each group for both male and female animals, along with the range of the score, indicates the consistency with which the results of these diets can be reproduced.

TABLE II. STATISTICAL EVALUATION OF CARIES SCORES

DIET	NUMBER OF ANIMALS	AVERAGE MOLARS* AFFECTED PER ANIMAL	TOTAL CARIES SCORE (AVERAGE PER ANIMAL)		TOTAL CARIES SCORE RANGE	STANDARD DEVIATION OF SCORE
A	12 ♂	3.3	0.9	0.9	0.0 - 2.3	0.21
	9 ♀	3.3	0.9		0.0 - 1.8	0.12
C	10 ♂	10.3	16.1	14.1	2.8 - 54.0	18.1
	12 ♀	9.7	12.5		2.8 - 39.3	10.1
D	5 ♂	8.8	13.7	12.6	6.5 - 22.5	5.5
	6 ♀	8.9	11.7		3.5 - 21.8	8.1
E	12 ♂	11.0	17.1	23.6	3.5 - 34.8	11.8
	10 ♀	10.7	31.5		2.0 - 107.0	35.6
F	14 ♂	7.1	2.8	2.3	0.8 - 7.0	1.6
	12 ♀	5.3	1.6		0.3 - 4.0	0.3

*Highest total possible is twelve.

The control diet (A) had the lowest total water-soluble carbohydrate and had a high fluoride content. Animals on this diet showed only a small caries score and had only three of a possible twelve molar teeth involved in carious lesions. The terminal weights of the animals on this diet were higher than any except those on the F diet.

TABLE III. PATTERN OF CA RIBUTION

DIET	MAXILLARY SCORE	MANDIBULAR SCORE	THIRD MOLAR		CERVICAL SCORE		TOTAL
			MAXILLARY SCORE	MANDIBULAR SCORE	FIRST MAXILLARY	FIRST MANDIBULAR	
A	0.7	0.2	0.3	0.05	0	0	0
C	7.5	6.6	4.8	3.8	0.2	1.0	1.5
D	8.3	4.3	5.0	2.4	0	0.1	0.1
E	10.4	13.2	7.1	4.8	0.4	2.6	3.6
F	1.4	0.9	0.7	0.2	0.1	0.3	0.4

The results obtained for the C diet, which contains sucrose and starch and may be considered as a control for Diets D and E, show strong evidence of caries destruction with 10.3 of the twelve molar teeth being involved in carious lesions and a total caries score of 14.9. The range and standard deviation values indicate that there is considerable variation in caries score from animal to animal on this diet. The terminal weights of these animals are somewhat less than those on the A and F diets.

Diet D is similar in composition to Diet C but contains 5 per cent sodium citrate. Animals on this diet, in comparison with those on the C diet, may exhibit a slight reduction in the general caries incidence, but at best the effect is slight.

The E diet, with sucrose as the principal constituent, shows an increase in the caries index in comparison with the C diet, which contains starch and sucrose. The statistical analysis of the results for this group may be somewhat misleading, since two female animals had greatly elevated scores and caused the group to present a higher index than would be predicted from the findings for the males of the same group.

The infant formula, Diet F, showed a very low score, only the control diet (A) having a lower score. This low score considered in relation to the number of teeth involved indicates a predominance of "beginning" caries, that is, a number of small superficial lesions.

DISCUSSION

The results obtained for Diets A, C, and E are generally consistent with those obtained by previous investigators for both rats and hamsters (Schweigert, Shaw, Phillips, and Elvehjem[7]; Mitchell, Chernausek, and Helman[6]; Shafer[8]; Keyes[2]; and Mitchell and Shafer.[4] The extent of the development of the carious process may be less than that found in earlier work. This is due to the experimental period of fifty-eight days, which is considerably less than the period usually employed (100 to 120 days). On the other hand, the animals were placed on the experimental diets at an earlier age (17 days) than the usual 30 days.

Sognnaes[9] has pointed out that for animals the relationship between dental caries and excessive consumption of carbohydrate may be in the nature of an unrecognized indirect influence on the quality of the offspring and its developing teeth, and that the effect may be cumulative through generations. Another influencing factor might be the fluoride content of the

stock diet on which the ha[illegible] olonies are usually maintained. The high fluoride content of these diets might cause a strain of caries-resistant animals to be produced which would require special diets for caries production.

Mitchell and Shafer[4] have shown that hamsters receiving 40 ppm of fluoride as NaF in a caries-producing diet were almost completely protected against caries regardless of the ages at which the fluoride was initiated. The low caries experience of the group on Diet A, usually considered a control diet in this type of experiment, probably is primarily due to the high fluoride content of the diet.

While the number of animals on the D diet is small, the results seem to be quite reproducible. There is a slight reduction in the number of teeth involved and in the total score when compared with the C diet, which contains the same amount of sucrose.

The particular infant formula used for Diet F showed a very low caries score, only a little above the control group (A). The total water-soluble carbohydrate in this diet was quite high (61.8 per cent), but was somewhat less than that for the cariogenic diets (C and E) (71 to 78 per cent). The carbohydrate material of this diet consisted of maltose, dextrins, and lactose (from the milk), whereas Diets C and E contained sucrose or uncooked starch. Uncooked starch differs greatly from cooked starch in its physical and chemical properties, and the cariogenicity of cooked or gelatinized starch may be quite different from that found with uncooked starch. In these experiments, as in most of the preceding experiments, uncooked starch has been used. Future work should indicate whether this difference is significant, since most starch ingested by human subjects has been cooked.

Although the particular infant formula tested in this work was found to be only slightly cariogenic under the particular experimental conditions employed, other sugars such as the common hydrolyzed starch syrups (''glucose'') may be quite different in their effects and require similar evaluation.

The infant formula, Diet F, differed also from the other diets in that a vitamin mixture, rather than lettuce, was used as a supplement. In view of the somewhat superior growth observed for the animals on this diet, the possibility is raised that the observed reduction in the extent of caries may have arisen from the presence of more adequate dietary supplements or from the increased amount of protein available.

As indicated in Table III, the general pattern of caries distribution is similar to that of earlier work. Within the rather large probable error, there seems to be no pronounced difference between the maxillary and mandibular molars. Most of the total caries score arises from the third molar, in all instances. There is an approximately linear relationship between total caries score and the total score for the maxillary third molar. Most of the cervical caries is found on the first molar. There seems to be no pronounced difference between the cervical score and the total score, since animals which have a high total score experience the most cervical lesions.

These results are consistent with earlier work in demonstrating that if the presence of carbohydrates in these diets is the primary reason for their cariogenicity, the type or physical character of the carbohydrate must be very important. Obviously, all dietary carbohydrates, then, cannot be considered to be cariogenic, and it is necessary to establish better the type and characteristics of cariogenic carbohydrates.

CONCLUSIONS

In general agreement with earlier work, it is shown that diets composed mainly of whole dry milk (30 per cent) and sucrose or sucrose with uncooked starch (66 per cent) cause the extensive development of caries in hamsters. These results were obtained in a shorter period than usual by placing 17-day-old hamsters on the experimental diets for fifty-eight days.

One common type of infant formula was tested for its cariogenic effect on hamsters. Although the total soluble carbohydrate was similar to that of the cariogenic diets, relatively little caries was produced at fifty-eight days. This may be the result of the specific types of carbohydrates included in this formula, or possibly the result of the better nutritional qualities of this diet.

The establishment of the conditions used for these experiments was accomplished through the advice and prior work of Dr. Joseph L. Volker. His advice and help, as well as that of Dr. C. E. Klapper and Dr. Sidney B. Finn, were of very great value for this investigation.

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STUDIES ON STANNOUS FLUORIDE AND OTHER FLUORIDES IN RELATION TO THE SOLUBILITY OF ENAMEL IN ACID AND THE PREVENTION OF EXPERIMENTAL DENTAL CARIES

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PREVIOUS work from this laboratory has shown that several compounds possess the ability to reduce the incidence of dental decay in the rat when furnished in the drinking water[1, 5] or in the diet.[2] Other work has involved a study of the effect of different compounds on the solubility of powdered dental enamel as determined by the method of Muhler, Boyd, and Van Huysen.[3] It was believed that the data from these studies would be significant in relation to the etiology and control of human dental caries. One group of workers, in considering the relationship between enamel solubility and human dental caries, wrote, "As an aid in selecting those (compounds) which are most likely to be beneficial, it would be logical to make a preliminary screening from tests on the surfaces of intact teeth and a second screening with tests on hamsters. After those substances which are more toxic than fluoride had been eliminated, the remainder would be substances which deserve clinical tests with human subjects."[17]

In a clinical study[11] on the effectiveness of sodium fluoride, two solutions with different hydrogen ion concentrations were used. The conclusion was reached that a 2 per cent solution at pH 7.0 is capable of significantly reducing the total interproximal and secondary dental caries incidence, while a 2 per cent solution at pH 3.5 is ineffective. However, Muhler and Day[4] showed that the pH of a sodium fluoride solution may have little or no effect on its ability to decrease the solubility of powdered dental enamel when only the decalcification values (those experimental values which are intended to be comparable to the effect of acids on the teeth after protection by fluorine) are used for comparative purposes. Therefore, a portion of this paper is devoted to an investigation of the caries-reducing effect of two different fluorides when placed in the drinking water at different hydrogen ion concentrations but at identical concentrations of fluoride.

The paper is an extension of in vivo work[1, 2, 5] dealing with the general topic of anticaries agents. Twelve different compounds were studied under various experimental conditions in order to determine the most effective one for use in decreasing dental caries in the rat. The study also extends the work[5] on the effect of differences in the source of fluorine on the amount of the element stored in the bones.

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TABLE 1

EFFECT OF DIFFERENT SOURCES OF FLUORINE IN THE DRINKING WATER ON THE INCIDENCE OF DENTAL CARIES IN RATS (SERIES 1)

COMPOUND	SEX	FLUORINE IN WATER (PPM.)	NUMBER OF ANIMALS	WEIGHT GAIN (GM.)	NUMBER OF LESIONS	EXTENT	REDUCTION (PER CENT)	DISTRIBUTION	
								LEFT (PER CENT)	RIGHT (PER CENT)
HF	Male	14	14	295	6.4 ± 0.90†	1.9	7	0 0 43	50 0 0
								0 100 93	93 100 0
	Female	14	14	155	5.6 ± 0.74	1.5	19	0 0 13	13 0 0
								0 50 88	88 50 0
NH_4BF_4	Male	11	18	287	6.6 ± 1.39	1.9	4	0 8 62	46 0 0
								0 93 93	93 100 0
	Female	11	11	169	7.2 ± 1.30	1.8	0	0 0 20	20 0 0
								0 100 100	100 100 0
NaF	Male	10	15	283	6.5 ± 1.73	1.7	6	0 0 20	30 10 0
								0 90 90	100 100 0
SnF_2*	Male	10	19	289	4.4 ± 1.90	1.5	36	0 0 43	28 0 0
								0 78 78	71 57 0
	Female	10	12	182	4.3 ± 1.13	1.6	40	0 0 17	33 0 0
								0 50 50	67 33 0
Control	Male	0.15	11	276	6.9 ± 1.14	2.1	0	0 50 60	65 45 0
								0 100 95	100 100 0
	Female	0.15	11	172	7.2 ± 1.44	2.2	0	0 35 60	70 45 0
								0 100 100	100 100 0

*I.U. Dess preparation.
†Standard error of the mean.

EXPERIMENTAL

In addition to the in vitro studies, three separate series of animal experiments were conducted comprising a total of 559 rats. In Series I, 125 animals of both sexes were used; Series II comprised 146 males; and Series III consisted of 288 males. All the animals were the Carl Wilson strain. They were placed on the supplements when 30 days old, but previous to this time the animals were on tap water (0.2 ppm fluorine), and they received the caries-producing diet (3.3 ppm fluorine) from the time they were old enough to eat. The duration of each experiment was 140 days, after which time the animals were sacrificed. The procedure for preparing the heads for examination and the handling of the animals has been previously reported.[1]

Some changes were made in the caries-producing diet. Previous to these studies the corn was ground in a local feed mill. The fluorine content of the diet prepared from this corn was 0.78 mg. per cent. For the experiments reported herein, shelled corn was ground in this laboratory when needed. One per cent of the particles was retained on 8 mesh, 35 per cent on 10 mesh, 37 per cent on 20 mesh, and 27 per cent passed 20 mesh. The fluorine content of the diet prepared from this was 0.33 mg. per cent. In all other respects the diet was the same as previously reported.[1]

Dental caries was counted in a manner identical to that previously reported,[1, 2] but in addition to counting the number of cavities, a factor intended to represent severity of the lesion was introduced in Series III. In a previous publication[5] the extent of the lesion was introduced. This value was intended to help express the degree of differences in the size of the lesions in each group. In this latter study the number of cavities and the extent were com-

TABLE II

EFFECT OF DIFFERENT SOURCES OF FLUORINE IN THE DRINKING WATER ON THE INCIDENCE OF DENTAL CARIES IN MALE RATS (SERIES II)

COMPOUND	FLUORINE IN WATER (PPM)	NUMBER OF ANIMALS	WEIGHT GAIN (GM.)	NUMBER OF LESIONS	EXTENT	REDUCTION (PER CENT)	DISTRIBUTION LEFT (PER CENT)			DISTRIBUTION RIGHT (PER CENT)		
NaF, (pH 3.7)	10	20	259	6.5 ± 0.93	1.67	7	0	0	53	47	0	0
							0	93	100	100	100	0
NaF, (pH 6.5)	10	20	264	7.1 ± 0.91	1.85	0	0	7	87	40	7	0
							0	87	100	100	87	0
SnF_2*	10	25	260	4.3 ± 0.60	1.44	39	0	0	30	20	0	0
							0	50	75	70	90	0
SnF_2†	10	20	271	4.5 ± 0.53	1.61	36	0	7	60	27	0	0
							0	100	80	67	100	0
Na_2SnF_6	10	25	258	5.4 ± 0.85	1.47	23	0	10	50	40	10	0
							0	90	85	90	80	0
Na_2SnF_6	30	20	256	5.7 ± 0.90	1.54	19	0	0	73	53	0	0
							7	67	100	93	60	0
Control	0.15	16	239	7.0 ± 1.29	1.88	—	0	10	50	40	0	0
							0	80	90	100	90	0

*General Chemical Co.
†I.U. Hensley preparation.

Table III

Effect of Different Sources of Fluorine and pH of Stannous Fluorine on the Incidence of Dental Caries in Male Rats (Series III)
(All Supplements Furnished Fluorine at 10 ppm)

Compound	Number of Animals	Weight Gain (Gm.)	Number of Lesions	Extent	Molars Affected (MA)	Severity Product (SP)	Reduction‡ (per cent	Distribution						
								Left (per cent)			Right (per cent)			
$NaF + SnF_2$	26	272	4.95 ± 0.77	1.43	4.04	7.08	32	0	0	46	17	0	0	
								0	75	87	83	92	0	
NaF (pH 3.7)	23	277	6.20 ± 0.80	1.55	4.63	9.60	16	0	0	47	42	0	0	
								0	85	100	95	95	0	
SnF_2* (fresh, pH 5.5)	24	253	5.85 ± 1.05	1.61	4.20	9.42	21	0	0	44	30	0	0	
								0	69	74	83	57	0	
$Cu(BF_4)_2$	23	226	6.32 ± 0.90	1.70	4.09	10.74	15	0	0	32	32	0	0	
								0	68	95	100	68	0	
$NaBF_4$	24	237	6.10 ± 1.32	1.47	4.65	8.98	18	0	0	50	30	0	0	
								0	90	100	100	80	0	
$SnF_2 + SnCl_2$	24	269	5.05 ± 1.11	1.44	4.10	7.27	32	0	0	40	25	0	0	
								0	80	85	85	95	0	
Na_2SiF_6	29	237	6.13 ± 0.85	1.70	4.36	10.42	17	0	0	47	42	0	0	
								0	74	100	95	79	0	
$Sn(BF_4)_2$	24	251	4.81 ± 0.71	1.44	3.68	6.93	35	0	0	42	37	0	0	
								6	58	90	84	54	0	
PbF_2	25	233	5.35 ± 0.64	1.46	4.10	7.81	28	0	0	40	30	0	0	
								0	80	80	90	90	0	
SnF_2† (fresh, pH 5.5)	28	263	5.20 ± 0.99	1.60	3.56	8.32	30	0	0	45	25	0	0	
								0	80	85	100	90	0	
SnF_2† (pH 3.7)	20	281	4.20 ± 0.50	1.43	3.50	6.00	43	0	0	25	15	0	0	
								0	80	100	80	80	0	
Control	18	231	7.40 ± 0.83	2.33	5.12	16.50	0.0	0	0	35	45	0	0	
								0	100	100	100	100	0	

*General Chemical Co.
†I.U. Hensley preparation.
‡Based upon comparison with the number of lesions, not severity product.

bined in one factor and called SP, severity product.* This value was obtained by multiplying the number of lesions in each animal by the extent for this same animal.

DATA

Tables I, II, and III indicate the caries status of the rats in the three series. The series were run at different times. Thus, it is not possible to make quantitative comparisons between animals in the different series in respect to the number of cavities, but differences in the percentage reduction of caries are meaningful. All three tables indicate the compounds used as supplements in the drinking water, the fluoride level at which the supplement was administered, the number of animals used, the increase in weight of the animals during the investigation, the number of lesions per animal, the extent of the lesions, the reduction in decay each compound produced as compared to distilled water and the distribution of the lesions in the different molar teeth.

TABLE IV

EFFECT OF DIFFERENT SOURCES OF FLUORINE IN THE DRINKING WATER ON THE DEPOSITION OF FLUORINE IN THE BONES (SERIES I)

COMPOUND	SEX	FLUORINE IN WATER (PPM)	MEAN WEIGHT OF DRY FEMUR (GM.)	MEAN WEIGHT OF ASHED FEMUR (GM.)	MEAN ASH (PER CENT)	MEAN FLUORINE CONCENTRATION (PPM)	MEAN TOTAL FLUORINE (MG. PER FEMUR)
HF	Male	14	0.7020	0.3780	53.8	1905	0.720
	Female	14	0.5315	0.2840	53.7	1900	0.540
NH_4BF_4	Male	11	0.6570	0.3422	52.2	1261	0.432
	Female	11	0.4775	0.2455	51.4	1038	0.255
NaF	Male	10	0.6915	0.3045	51.4	1455	0.438
SnF_2*	Male	10	0.7085	0.3660	51.8	1162	0.426
	Female	10	0.4915	0.2590	52.7	1185	0.307
Control	Male	0.15	0.6110	0.3166	51.8	250	0.079
	Female	0.15	0.4875	0.2400	51.6	327	0.078

*I.U. Dess preparation.

TABLE V

EFFECT OF DIFFERENT SOURCES OF FLUORINE IN THE DRINKING WATER ON THE DEPOSITION OF FLUORINE IN THE BONES OF MALE RATS (SERIES II)

COMPOUND	FLUORINE IN WATER (PPM)	MEAN WEIGHT OF DRY FEMUR (GM.)	MEAN WEIGHT OF ASHED FEMUR (GM.)	MEAN ASH (PER CENT)	MEAN FLUORINE CONCENTRATION (PPM)	MEAN TOTAL FLUORINE (MG. PER FEMUR)
NaF (pH 3.7)	10	0.7450	0.3663	49.3	1610	0.603
NaF (pH 6.5)	10	0.7387	0.3643	49.2	1770	0.647
SnF_2*	10	0.7634	0.3780	49.5	1683	0.636
SnF_2†	10	0.7027	0.3726	52.8	1274	0.470
Na_2SnF_6	10	0.7157	0.3593	50.2	2054	1.154
Na_2SnF_6	30	0.6140	0.2960	48.0	4050	0.707
Control	0.15	0.6810	0.3560	52.3	269	0.095

*General Chemical Co.
†I.U. Hensley preparation.

*We wish to acknowledge the helpful suggestions of Arthur W. Radike in setting up a more critical method of evaluating the differences in the various experimental groups.

Tables IV, V, and VI refer to the amount of fluorine stored in the body. The data were obtained by analyzing the right femur as a representative part of the calcified tissues. All of these tables show the sex, mean weight when sacrificed, mean weight of the dry and ashed femur, mean ash, mean fluoride concentration, and the total amount of fluorine.

TABLE VI

EFFECT OF DIFFERENT SOURCES OF FLUORINE IN THE DRINKING WATER ON THE DEPOSITION OF FLUORINE IN THE BONES OF MALE RATS (SERIES III) (ALL SUPPLEMENTS FURNISHED FLUORINE AT 10 PPM)

COMPOUND	MEAN WEIGHT OF DRY FEMUR (GM.)	MEAN WEIGHT OF ASHED FEMUR (GM.)	MEAN ASH (PER CENT)	MEAN FLUORINE CONCENTRATION (PPM)	MEAN TOTAL FLUORINE (MG. PER FEMUR)
$NaF + SnF_2$	0.7315	0.3680	50.2	2136	0.787
NaF	0.7688	0.3860	53.4	2021	0.763
SnF_2* (fresh, pH 5.5)	0.7270	0.3770	51.8	1990	0.713
SnF_2† (fresh, pH 5.5)	0.7807	0.3546	45.6	2174	0.776
SnF_2† (pH 3.7)	0.7115	0.3734	52.6	1478	0.564
$Cu(BF_4)_2$	0.6260	0.2950	46.7	1910	0.598
$NaBF_4$	0.8677	0.3823	47.9	1734	0.648
$Sn(BF_4)_2$	0.6430	0.3032	52.3	1887	0.648
$SnF_2 + SnCl_2$	0.7696	0.3473	45.1	1925	0.645
Na_2SiF_6	0.7063	0.3274	46.5	2010	0.655
PbF_2	0.6590	0.3534	50.7	1405	0.483
Control	0.8613	0.3950	46.5	268	0.104

*General Chemical Co.
†I.U. Hensley preparation.

In an attempt to get representative bone samples from each series, as well as from each animal, different methods of sampling were tested. Representative animals receiving the various fluoride supplements were sacrificed and the long bones were removed and ashed. The data indicated that the concentration of fluorine in different long bones of the same animal was

TABLE VII

THE AMOUNT OF FLUORINE STORED IN THE HEAD AND IN THE SHAFT OF THE FEMUR OF THE RAT AFTER FURNISHING VARIOUS FLUORIDES IN THE DRINKING WATER FOR 140 DAYS (ALL SUPPLEMENTS FURNISHED FLUORINE AT 10 PPM)

COMPOUND	MEAN FLUORINE CONCENTRATION		MEAN TOTAL FLUORINE	
	HEAD (PPM)	SHAFT (PPM)	HEAD (MG.)	SHAFT (MG.)
$NaF + SnF_2$	2136	1740	0.787	0.642
SnF_2* (pH 5.5)	1890	1340	0.706	0.500
NaF (pH 3.7)	2021	1990	0.743	0.737
PbF_2	954	620	0.354	0.231
$Cu(BF_4)_2$	1910	1576	0.598	0.410
$NaBF_4$	1555	1170	0.665	0.496
Na_2SiF_6	2225	1870	0.719	0.604
$Sn(BF_4)_2$	2500	1720	0.960	0.660
SnF_2† (pH 5.5)	2220	1935	0.787	0.687
SnF_2† (pH 3.7)	1478	1009	0.564	0.384
$SnF_2 + SnCl_2$	2166	1720	0.732	0.577
Control	263	287	0.104	0.110

*General Chemical Co.
†I.U. Hensley preparation.

TABLE VIII

COMPARISON OF THE EFFECTIVENESS IN DECREASING THE SOLUBILITY OF POWDERED ENAMEL IN ACID WITH THE EFFECTIVENESS IN DECREASING THE INCIDENCE OF DENTAL CARIES IN RATS

COMPOUND	FLUORINE CONCENTRATION (μG PER ML.)	pH	REDUCTION OF ENAMEL SOLUBILITY (PER CENT)	REDUCTION OF CARIES (PER CENT)
$NaF + SnF_2$	50	3.9	55.2	
	100	4.1	65.3	32
	300	4.4	75.0	
	500	4.5	79.8	
NaF	50	3.7	50.6	
	100	3.7	54.2	16
	300	3.7	60.3	
	500	3.7	60.9	
$Cu(BF_4)_2$	50	3.5	44.6	
	100	2.3	47.3	
	300	3.1	56.5	15
	500	3.0	60.0	
$NaBF_4$	50	4.0	28.3	
	100	3.8	37.6	18
	300	3.5	43.4	
	500	3.3	47.3	
$SnF_2 + SnCl_2$	50	3.1	67.7	
	100	2.9	79.6	
	300	2.7	92.5	32
	500	2.4	94.0	
Na_2SiF_6	50	3.3	51.3	
	100	3.2	56.5	
	300	3.2	60.5	17
	500	3.2	60.2	
$Sn(BF_4)_2$	50	3.0	47.2	
	100	2.8	55.0	
	300	2.4	72.5	35
	500	2.2	78.0	
PbF_2	50	4.9	52.4	28
	100	5.0	62.5	
SnF_2 (fresh)	50	5.5	59.4	
	100	5.5	70.0	
	300	5.5	91.4	30
	500	5.5	94.5	
SnF_2	50	3.7	85.9	
	100	3.6	89.6	43
	300	3.4	93.4	
	500	3.3	96.5	
HF	50	3.1	54.0	
	100	3.0	56.0	13
	300	2.7	60.8	
	500	2.4	66.8	
NH_4BF_4	50	4.7	15.3	
	100	4.2	23.6	
	300	3.8	37.5	2
	500	3.6	40.7	
Na_2SnF_6	50	3.6	51.0	
	100	3.3	55.0	
	300	3.3	62.8	17
	500	3.3	63.4	
Na_2SiF_6	50	6.0	44.0	
	100	6.0	47.3	
	300	6.0	52.0	
	500	6.0	55.0	
NaF	50	6.5	46.4	
	100	6.5	48.8	10
	300	6.5	54.5	
	500	6.5	57.2	

approximately equal. For this reason the femur was chosen for analysis in this and subsequent work, due mainly to its size and the convenience in removing it.[6] Table VII is included to show that there is considerable difference between the head and the shaft in the concentration of fluorine.

A great amount of the early work dealing with the action of fluorides in reducing dental caries was an outgrowth of in vitro studies using powdered dental enamel.[3, 7-9] In some instances, such as in the use of sodium fluoride, a good correlation has been established between effects on enamel solubility and experimental dental caries. Realizing that results from animal experimentation may not be entirely comparable to those from clinical investigations, a study was made of the relation of enamel solubility to the ability of these same compounds to reduce the incidence of caries in the rat. Table VIII demonstrates the findings. It indicates the compound used to protect the enamel, the effect that various concentrations of the compounds have in reducing powdered enamel solubility, the pH of the solutions used in treating the enamel, and the reduction in decay found in the experimental animal when receiving these same compounds as supplements to their drinking water. The extensive data in this table are summarized in Table IX.

TABLE IX

SUMMATION OF COMPARISON OF EFFECTIVENESS ON ENAMEL SOLUBILITY WITH THE EFFECT ON THE INCIDENCE OF DENTAL CARIES IN THE RAT

COMPOUND	SOLUBILITY REDUCTION SUMMATION	REDUCTION IN INCIDENCE OF CARIES (PER CENT)
SnF_2(pH 3.7)	365	43
SnF_2 + $SnCl_2$	338	32
SnF_2(pH 5.5)	315	30
NaF + SnF_2	275	32
$Sn(BF_4)_2$	253	35
Na_2SiF_6	229	17
NaF(pH 3.7)	226	16
$Cu(BF_4)_2$	208	15
$NaBF_4$	157	18
NH_4BF_4	117	2

RESULTS AND DISCUSSION

Table I indicates that stannous fluoride is the only supplement used in Series I that effectively reduced the incidence of caries when the fluoride level of the supplements was about 10 ppm. This reduction occurred in both sexes to about the same degree. Ammonium fluoroborate and sodium fluoride had no effect at the concentration used in reducing the decay rate. It is of interest to note that on an average the hydrofluoric acid reduced the caries incidence by 19 per cent in the females, while only by 7 per cent in the males. No explanation is offered except that this is probably a fortuitous difference. The animals given hydrofluoric acid received a greater fluoride concentration than those receiving sodium fluoride, but previous work in this laboratory[1, 5] and elsewhere[10] has indicated that at a level of 10 ppm fluorine, as sodium fluoride, little if any, protection is afforded the animals. These data, therefore, are in keeping with previous findings. The control animals not only had

the greatest number of cavities, but also the highest severity index, while those animals receiving stannous fluoride had the fewest cavities and also the lowest severity as illustrated by the values for extent of the lesions. No outstanding differences were evident in the distribution of the cavities in the various groups. The mandibular teeth were affected to a greater extent than the maxillary teeth in all cases.

The results in Series II, Table II, are of interest due to previously published in vitro studies[4] and in vivo clinical findings[11] based on the use of sodium fluoride. It has been suggested[12, 13] that the lower the pH of the sodium fluoride, the more effective this solution is in reducing powdered enamel solubility. This was not substantiated when sodium fluoride, at two different hydrogen ion concentrations, was tested clinically. However, the clinical study should be considered as a doubtful one, since only forty-seven adult students were used. Additional in vitro studies by Muhler and Day[4] also failed to support the original hypothesis if only the decalcification, and not the treatment experimental values, are compared. The data obtained in this animal work indicate that difference in pH of the fluoride has little, if any, effect on the ability of sodium fluoride to reduce experimental decay when the fluoride level is approximately 10 ppm but, since the level has previously been shown not to have a pronounced effect on rat caries, it would be of value to test the effect of pH at a higher fluoride level. The fact that sodium fluoride at this fluoride level failed to reduce the incidence of caries, even at a neutral pH, is to be expected from previous work.[1, 5, 10] The indication is that a 7 per cent reduction occurs when the same stoichiometric equivalent of fluorine is given but at pH 3.7 does not appear to be significant, although the extent of the lesions in this group was certainly smaller than in the group receiving the less acidic solution. Additional work at higher fluoride levels is indicated to clarify this point.

It is to be noted that the only supplements showing an ability to reduce caries in Series II are those which contained tin. Stannous fluoride from two different sources was tested in this series, and little if any difference was noted in the percentage reduction, but some difference was indicated regarding the severity of the lesions. The approximately 40 per cent reduction noted in this series is similar to that found in Series I and very similar to previous experience in this laboratory based on the use of this compound.[1, 2, 5] The results of administering sodium hexafluorostannate are interesting, for there appears to be no increase in caries prevention with increasing amounts of fluorine. The animals receiving 10 ppm fluorine as sodium hexafluorostannate had a 23 per cent reduction, while those animals that received three times this amount of fluorine from the same compound had about the same degree of protection. This finding is not in keeping with the results of other experiments[1, 2, 5] wherein increasing the fluorine concentration of both sodium fluoride and stannous fluoride increased the protection against dental caries.

The reduction in caries normally experienced by giving stannous fluoride (approximately 40 per cent) is definitely decreased when the pH of the

drinking water supplement is increased to 5.5. These data are seen in Table III, which is Series III of this study. When freshly prepared stannous fluoride solution was given and the pH was approximately 3.0, a 60 per cent reduction in caries was reported.[5] When it was fresh, but adjusted to a pH of 5.5, the effectiveness decreased by approximately 50 per cent. When fresh stannous fluoride stock solution was made about every week or two, and working dilutions were made from this solution (pH 3.7), there was a 43 per cent reduction. This result is not at all like that which is found when sodium fluoride is given under similar conditions (Series II), for it has been demonstrated that the pH of sodium fluoride solutions is relatively unimportant in protecting the rats' teeth against caries when the fluoride level is quite low.

In the results of experiments in Series III, it appeared that sodium fluoride has some effect in reducing caries at a low pH, for a 16 per cent reduction was found. This is not in keeping with the results of Series II, for little difference was noted in caries incidence regardless of the pH of the sodium fluoride solution. One explanation of this might be that in this series more extensive caries was present in the control group, and perhaps, since the number and extent of the carious lesions was somewhat greater in this group, the data for the supplement in general are somewhat exaggerated.

Previous experience with small concentrations of borates (Series I) indicated that no protection was afforded the experimental animal against dental caries. In Series III copper fluoroborate and sodium fluoroborate behaved similarly, but stannous fluoroborate showed marked protective ability. Again, of the borate compounds so far studied, the only one demonstrating any degree of caries reduction contains a tin moiety.

Further proof that the tin component increases the protection against caries is indicated by the data obtained when giving sodium fluoride with stannous fluoride. In this combination, each compound contributed 5 μg of fluorine per milliliter, making the total fluoride concentration approximately 10 ppm. In this instance, the tin moiety must have influenced the action of the fluoride, for 5 μg of fluorine per milliliter as stannous fluoride alone would not produce a reduction of this magnitude, and previous experience has indicated little protection even at 10 μg fluorine per milliliter with sodium fluoride. Assuming, then, that stannous tin activates the fluorine in some manner, it is not unreasonable to suppose that if one increased the tin concentration a still greater degree of protection would occur. This idea was tested by adding stannous chloride to stannous fluoride. No additional protection was evident.

Sodium silicofluoride is widely regarded as equivalent to sodium fluoride for the purpose of fluoridizing municipal water supplies. This is justified by some on the theoretical grounds that any inorganic fluoride compound will dissociate almost completely at the concentration used for fluoridation, furnishing fluoride ions.[14, 15] Evidence as to the metabolic similarity between the two compounds is found in the study demonstrating that approximately the same amount of fluoride is stored in the animal body regardless of whether

sodium fluoride or sodium silicofluoride is administered in the drinking water.[16] The data presented here indicated that sodium silicofluoride may have about the same caries-reducing ability as sodium fluoride, although the effect is so slight in both instances that quantitative appraisal of the results is scarcely possible. However, whether or not it is significant, the severity of the lesions in the sodium fluoride animals was certainly much less than in those receiving sodium silicofluoride; in fact, it was similar in degree to caries in the animals receiving copper fluoroborate. The animals receiving the latter compound appeared to have little protection, either in number of cavities or in extent of the lesion.

In vitro experiments suggested that lead fluoride is superior to sodium fluoride in reducing the solubility of powdered dental enamel;[3, 8, 17] yet, clinically, lead fluoride failed to reduce the incidence of decay.[18] When lead fluoride was fed at a level of approximately 10 μg fluorine per milliliter in the drinking water, a good degree of reduction in experimental decay resulted. This reduction in decay was evidenced also by the fact that the severity of the lesions was as low as in any case previously demonstrated. This apparent discrepancy between in vitro work and clinical studies might tend to discredit in vitro solubility studies, or indicate a fallacy in certain instances in in vivo experiments with the rat. However, it is felt that if the solubility of lead fluoride were higher a reduction similar to or greater than that achieved with sodium fluoride would have been experienced clinically. Perhaps lead, like tin, increases the effectiveness of fluorine and when used at the concentration of this experiment, sufficient amounts were available to account for the reduction observed. This strengthens the evidence that certain cations, as well as the fluorine, should be considered.[5, 18]

The modified method of recording caries incidence and severity is indicated in Table III in order that the results may be compared with other methods. The data comprising the modified method indicate the molars affected, the severity of the lesions as compared with the animals receiving no supplement (indicated as SP, severity product), and the percentage reduction for each supplement based upon this severity product number. It is interesting that the modified procedure of counting caries indicates the same general pattern as the original method. For example, stannous fluoride at pH 3.7 allowed the smallest number of carious lesions and the lowest extent value as indicated by the older method of counting caries. As compared with the modified method, it had the fewest number of molars affected and the severity product was the smallest. There are a few minor exceptions to this generalization. For example, stannous fluoride at pH 5.5 appeared to be the fifth best compound as determined by the older method, but the modified method indicates that under these conditions the compound is somewhat better in its ability to reduce experimental decay in the rat. In general, then, this newer method appears to corroborate the older method, but it does not seem to be particularly superior. More exact criteria for the evaluation of caries are being sought in this laboratory.

In Table IV analytical data are given for fluorine storage in the rat following the administration of the supplements indicated in the first column. The caries picture for these compounds is found in Table I and already has been discussed. It has been mentioned that hydrofluoric acid had a greater effect than sodium fluoride in reducing experimental decay. This difference was also found in the amount of fluorine stored in the body, but the concentration in the drinking water was about 40 per cent greater. Table IV shows that the males given hydrofluoric acid stored about 0.7 mg. of fluorine per femur, while the animals on sodium fluoride stored about 0.4 mg. This indicates that these compounds have equal effects when they furnish equal amounts of fluorine.

The data regarding the storage of fluorine from stannous fluoride corroborate work published previously from this laboratory.[5] Less fluorine was stored when stannous fluoride was furnished than when the supplement was sodium fluoride.

The findings concerning ammonium fluoroborate are significant because they support the opinion that the criterion of usefulness of a fluoride should not be solely its ability to promote the deposition of fluorine in the skeletal system. When ammonium fluoroborate was furnished at the same fluoride level as either sodium fluoride or stannous fluoride, it resulted in the storage of approximately the same amount of fluorine as the data showed for stannous fluoride. These findings may not be interpreted on the basis that the effectiveness in preventing caries parallels the effect on storage of fluorine, as one author has suggested,[16] because the animals receiving ammonium fluoroborate had no reduction in decay, while those receiving the same amount of fluorine as stannous fluoride had about 40 per cent reduction.

Table V summarizes the analytic data on the storage of fluorine in the rat after the administration of various compounds of fluorine to animals in Series II. The experimental results concerning the ability of these compounds to reduce the incidence of caries are given in Table II. These data indicated that there is no difference between acidulated sodium fluoride and approximately neutral sodium fluoride in the ability to reduce the incidence of decay. There was no real difference between the two in the storage of fluorine in the skeleton. This agrees with previous work from this laboratory, in which it was demonstrated that a change in the pH of the sodium fluoride solution failed to alter its ability to protect powdered dental enamel.[4]

Further data are presented in this series of experiments, demonstrating the fallacy of trying to predict the ability of a compound to reduce dental caries solely on the criterion of its effect in depositing fluorine in the body. Sodium hexafluorostannate at a level of 10 μg fluorine per milliliter reduced the incidence of caries by 23 per cent. When three times this amount of fluorine was fed as sodium hexafluorostannate no difference was noticed in the incidence of caries, although 50 per cent more fluorine was stored in the skeleton. Further data indicating the complexity of the whole picture were found in the case of the two stannous fluoride compounds used in Series II

(Table V). Stannous fluoride from one source resulted in the storage of approximately 0.64 mg. of fluorine in the femur, while another preparation of stannous fluoride compound caused the storage of 0.47 mg. Still, the caries incidence was about the same (approximately 40 per cent reduction) in both cases. Previous data[5] had indicated that stannous fluoride, at the same stoichiometric fluoride level as sodium fluoride, promoted the storage of considerably less fluorine in the skeleton. One compound used in this series indicated this again, while another indicated the level stored to be about similar to that deposited as sodium fluoride.

Also, the data presented in Table VI indicate that some preparations of stannous fluoride cause the storage of considerably less fluorine than occurs when sodium fluoride is given at the same fluoride level. Where stannous fluoride was administered at a fluoride level of 10 ppm, approximately 0.56 mg. of fluorine was stored, while sodium fluoride at approximately the same level caused the storage of 0.76 mg. Again, the compound causing the storage of the greatest amount of fluorine had the least effect in reducing dental caries (Table III).

The data in Table III show that when the pH of the stannous fluoride solution is increased to 5.5 the ability of the compound to reduce caries is decreased. Also, the data indicate that considerably more fluorine is stored in the femur when stannous fluoride is at the higher pH. Perhaps this may be explained on the basis that sodium fluoride is formed when sodium hydroxide is added to stannous fluoride, and that the former compound promotes greater storage of fluorine.

Lead fluoride was superior to sodium fluoride in its ability to reduce the incidence of dental decay, as shown in Table III, although the amount of fluorine deposited in the femurs was less than in rats given sodium fluoride. In fact, the amount of fluorine stored was the lowest for any compound studied in Series III.

The amount of fluorine stored in the femur as the result of feeding the various fluoroborates used in Series III is of interest. Ammonium fluoroborate (Table IV) caused the storage of approximately the same amount of fluorine as did sodium fluoride, while both failed to reduce caries to any appreciable extent. Tin fluoroborate and sodium fluoroborate resulted in the storage of about the same amount of fluorine, yet the tin compound was twice as effective in reducing caries. The copper compound caused the storage of somewhat less fluorine than either the tin or the sodium borate and considerably less than sodium fluoride, although its effectiveness in reducing caries was of the same order as sodium fluoride or sodium fluoroborate. Of the various fluoroborates so far examined, the only one demonstrating an ability to reduce caries is the one which contains a tin moiety.

It has been reported that sodium silicofluoride and sodium fluoride result in the storage of similar amounts of fluorine in the rat.[16] The data in Table VI corroborate this work. The caries picture for these two salts was almost identical, although the severity of the lesions was somewhat greater in the silicofluoride group (Table III).

TABLE X

A COMPARISON OF THE EFFECTIVENESS OF BUFFERED SOLUTIONS OF STANNOUS FLUORIDE AND SODIUM FLUORIDE IN REDUCING THE SOLUBILITY OF POWDERED DENTAL ENAMEL (100 MG. PER SAMPLE)

PROTECTING COMPOUND	BUFFERED PROTECTING SOLUTION		PHOSPHORUS DISSOLVED FROM ENAMEL								CALCIUM DISSOLVED FROM ENAMEL							
	FLUORIDE CONCENTRATION (ppm)	pH	PROTECTING TREATMENT	DECALCIFICATION TREATMENT (MG. PER SAMPLE)							PROTECTING TREATMENT	DECALCIFICATION TREATMENT (MG. PER SAMPLE)						
				1	2	3	4	5	6	7		1	2	3	4	5	6	7
SnF_2	10	3.7 (Pht)	0.39	1.09	1.26	1.28					0.99	2.44	2.78	2.83				
NaF	10	3.7 (Pht)	0.52	1.28	1.38	1.41					1.29	2.69	2.90	3.06				
SnF_2	50	3.0 (Pht)	0.15	0.58	0.90	1.02	1.05	1.00	1.02	1.02	0.67	1.38	2.00	2.23	2.39	2.34	2.32	2.30
NaF	50	3.0 (Pht)	0.98	1.05	1.19	1.14	1.16	1.16	1.11	1.05	2.18	2.34	2.74	2.55	2.66	2.58	2.51	2.42
SnF_2	100	3.1 (none)	0.04	0.23	0.75	1.00	1.11	1.16	1.19	1.20	0.21	0.60	1.64	2.08	2.34	2.48	2.42	2.37
NaF	100	3.1 (Pht)	0.97	0.77	1.08	1.19	1.26	1.22	1.26	1.20	2.09	1.77	2.46	2.55	2.69	2.69	2.67	2.46
SnF_2 (fresh)	1000	3.1 (none)	0.06	0.06	0.34	0.75	0.93	1.03	1.10	1.10								
SnF_2 (aged)	1000	2.2 (none)	0.84	0.74	1.03	1.14	1.16	1.24	1.21	1.20								
NaF	1000	3.1 (Pht)	1.12	0.04	0.10	0.38	0.59	0.69	0.78	0.81								
SnF_2 (fresh)	2.0%	2.8 (none)	0.04	0.01	0.06	0.42	0.74	0.90	1.00	1.04								
NaF	2.0%	2.8 (Pht)	1.02	0.16	0.08	0.22	0.39	0.54	0.63	0.67								

Pht = Phthalate buffer system.

There were some differences in the amount of fluorine stored in the femurs when a stannous chloride-stannous fluoride mixture was compared with a sodium fluoride-stannous fluoride mixture, although the reduction in caries was identical in both instances. It appeared that the animals receiving the stannous chloride-stannous fluoride mixture stored less fluoride than those receiving the sodium fluoride-stannous fluoride supplement, even though the fluoride concentration was the same in both instances. Additional studies should be made concerning the effect of the cation on the storage of fluorine in the skeleton.

The data in Table VII indicate that the head of the femur contains the greatest amount of fluorine. This is true in all instances except in those animals receiving no fluoride supplement. This indicates the need for caution in the determination of fluorine in the bones and in the interpretation of analytic data on this subject.

Table VIII is intended to present a comparison between the effect of different compounds on the reduction in enamel solubility at various fluoride concentrations and the ability of these same compounds in inhibiting dental caries. The table lists the compounds, the fluoride concentration used in the in vitro study, the pH of the solutions, the per cent reduction in enamel solubility, and the per cent reduction these compounds demonstrated in inhibiting the incidence of decay. In order to make a comparison between these two variables more easily, Table IX was constructed. The summation number was derived by adding all the values obtained on reduction in enamel solubility in Table VIII for each compound. The ability of these compounds to reduce the incidence of decay is also indicated for comparative purposes. Therefore, these whole numbers embody two ideas: first, they represent the difference between the per cent reduction at the lowest and highest fluoride level and, second, the size of the number indicates the ability to protect powdered dental enamel from being dissolved in a weak organic acid. Obviously, the higher the number, the greater is its ability to reduce enamel solubility.

A very interesting relationship is given in Table IX. The compound having the greatest numerical value, stannous fluoride (pH 3.7) also has been demonstrated to have the greatest ability to reduce the incidence of caries. Excellent correspondence is seen throughout this chart, except in the instance of sodium fluoroborate. Its numerical value of 157 indicated that its protectiveness was very low, but it reduced the incidence of decay in the rat by 18 per cent. Ammonium fluoroborate failed to reduce the incidence of decay in the rat and had the lowest solubility figure of those tested. This preliminary work indicates, therefore, that some reliance can be placed upon the ability of a compound to reduce enamel solubility and subsequently, if such technics indicate favorable results, also reduce caries in the rat. Obviously, it is not intended to predict the ability of every possible fluoride or nonfluoride compound, for exceptions undeniably will occur, but it is stressed that a useful tool is available to screen large numbers of compounds in a relatively easy and simple manner before spending considerably more time in animal or human experimentation.

In the work previously published from this laboratory[4] it was shown that little difference exists in the ability of sodium fluoride or stannous fluoride to protect powdered enamel against solubility in acid regardless of the pH of the solution used, provided that only the decalcification values (Table X) were compared. Since buffered protecting solutions were not used in any of the previous studies, additional work was done.

Table X shows the ability of buffered solution of sodium fluoride and stannous fluoride to reduce the solubility of 60-mesh powdered dental enamel. The results confirmed the previously published work[3, 4] which showed that with stannous fluoride considerably less phosphate is removed during the protection of the enamel than in the case of sodium fluoride. At the higher fluoride levels scarcely any phosphate is lost by using stannous fluoride, while increasingly large amounts are lost when sodium fluoride is used. The amount of calcium removed paralleled the values for phosphorus. If the enamel is protected once with the respective buffered solutions and decalcified repeatedly with a fresh decalcifying solution each time, increased effectiveness occurs for stannous fluoride at fluoride levels of 10, 50, or 100 μg per milliliter. However, at a concentration of 1,000 μg per milliliter repeated decalcifications of the treated enamel gave results which indicated that only freshly prepared solutions of stannous fluoride are superior to sodium fluoride.

CONCLUSIONS

1. Several different compounds of tin possess the ability to reduce the incidence of dental caries in the rat when given in the drinking water at a fluorine level of only 10 μg per milliliter.

2. Knowledge of the level of fluorine administered in the drinking water or stored in the skeletal system does not provide a safe basis for judging the degree of caries reduction which may occur.

3. Increasing the pH of stannous fluoride in solution decreases its effectiveness in reducing dental caries in the rat. It has been noted that freshly prepared solutions of stannous fluoride at approximately pH 3.0 result in the storage of less fluoride in the skeletal system than does sodium fluoride. However, increasing the pH of the stannous fluoride solution also increases the storage of fluorine in the skeletal system to values similar to those for sodium fluoride.

4. The effect of fluorides on enamel solubility seems to parallel their effect in reducing the incidence of dental caries in the rat.

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EFFECTS OF SEVERAL FLUORIDE REAGENTS ON THE SURFACE STRUCTURE OF POWDERED DENTAL ENAMEL

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SEVERAL investigators who have employed electron diffraction and x-ray diffraction methods for the study of the effects of sodium fluoride treatment on dental enamel have reported a transformation of the basic apatite structure to a structure of calcium fluoride.[1-3] It has been found[3] that there is no observed transformation after twenty-four hours' treatment when the pH of the fluoride solution is near neutral, but that the transformation proceeds readily at pH 4 even for treatments with time intervals as short as five minutes, and even more readily at a pH of 3. A combined electron diffraction and x-ray diffraction approach has shown[3] that the formation of calcium fluoride occurs predominately upon the surface of the powdered dental enamel particles. Similar studies[3] also revealed that treatments with hydrofluoric acid are essentially similar to those with sodium fluoride at low pH.

In the present study, powdered dental enamel portions were treated with solutions of potassium fluoride, sodium silicofluoride, sodium monofluorophosphate, stannous fluoride, and stannous chloride under various controlled conditions. Electron diffraction and x-ray diffraction patterns were taken of the samples before and after treatment.

PROCEDURE

The procedure employed was the same as that reported by us in an earlier paper.[3] Portions of 300 mesh enamel, weighing about 100 mg. each, were treated with 10 ml. portions of the appropriate solutions. The suspension was shaken in a mechanical shaker for the designated period of time, intermittently for treatments of twenty-four hours and longer, and continuously for the shorter periods of treatment. The enamel was then separated by filtration and washed three times with small portions of fluoride-free water. Electron diffraction patterns were taken with the conventional electron diffraction setup, using an RCA type EMU electron microscope. The x-ray diffraction patterns were taken photographically on a Norelco x-ray diffraction unit. Each pattern was identified by direct comparison with patterns of known substances.[3] Estimates of intensities of diffraction lines were made visually to provide a semiquantitative assay of patterns which revealed lines of more than one substance.

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RESULTS AND DISCUSSION

The data obtained on samples treated with potassium fluoride are summarized in Table I. The change from an apatite structure to one of calcium fluoride apparently did not take place at all in a solution near neutrality. At a pH of 4, the calcium fluoride lines began to develop in the electron diffraction pattern but not in the x-ray pattern. At a pH of 3, the electron diffraction patterns reveal only calcium fluoride, while both substances are indicated in appreciable amount by the x-ray pattern. The results at a pH of 2 are essentially similar to those at a pH of 3, except that the apatite lines are relatively much weaker in the x-ray patterns. From these results, three conclusions may be drawn concerning treatment of powdered dental enamel with potassium fluoride: the original apatite structure is transformed to a structure of calcium fluoride; the rate of this change increases markedly as the pH is lowered; the change develops predominately upon the surface of the particles. These results are very similar to those reported elsewhere[3] for treatments with sodium fluoride, although the rate of change appears definitely greater with sodium fluoride than with potassium fluoride. The close similarity, however, is very reasonable, and it further substantiates the concept that the anion is the significant part of the reagent when transformation from the apatite structure to one of calcium fluoride is being considered.

TABLE I. DATA ON POWDERED ENAMEL SAMPLES TREATED WITH POTASSIUM FLUORIDE (CONCENTRATION = 3,000 γ F IN EACH CASE)

TIME OF TREATMENT (HOURS)	pH	ELECTRON DIFFRACTION PATTERN	X-RAY DIFFRACTION PATTERN
½	6.4 (unadjusted)	Apatite	—
1	6.4	Apatite	Apatite
24	6.4	Apatite	Apatite
½	4.0	Apatite	—
1	4.0	Apatite + trace CaF_2	Apatite
24	4.0	Apatite + trace CaF_2	Apatite
½	3.0	CaF_2	—
1	3.0	CaF_2	CaF_2 + apatite
24	3.0	CaF_2	CaF_2 + apatite
½	2.0	CaF_2 + apatite	—
1	2.0	CaF_2	CaF_2 + little apatite
24	2.0	CaF_2	CaF_2 + little apatite

Typical data obtained on samples treated with sodium silicofluoride are summarized in Table II. Most of the data represent several repeated experiments. The data summarized in this and the other tables in this report represent approximately 200 samples of powdered enamel which have undergone treatment, on most of which x-ray patterns have been obtained and on all of which electron diffraction patterns have been repeated at least five times. Additional experiments were conducted with sodium silicofluoride at a pH of 6, with the apatite structure persisting, and at various pHs with a lower concentration of the solute, again with the apatite structure persisting. From these data it is evident that this reagent also causes a change of apatite

structure to a structure of calcium fluoride, and that the rate of the change increases as the pH is lowered. The transformation is revealed at least as readily by the x-ray pattern as by the electron diffraction pattern. This observation indicates that the change is not so highly localized upon the surfaces of the particles, as it is in the cases of the alkali fluorides already discussed, but rather that it develops to an appreciable extent more deeply within the enamel particles. There are two distinctive characteristics of the transformation as it is brought about by sodium silicofluoride: the rate is slower than with the alkali fluorides at comparable pH values; the change develops somewhat within the particles rather than so predominately upon the surfaces.

TABLE II. DATA ON POWDERED ENAMEL SAMPLES TREATED WITH SODIUM SILICOFLUORIDE (CONCENTRATION = 3,000 γ F IN EACH CASE)

TIME OF TREATMENT (HOURS)	pH	ELECTRON DIFFRACTION PATTERN	X-RAY DIFFRACTION PATTERN
1	3.3 (unadjusted)	Apatite + little CaF_2	CaF_2 + apatite
24	3.3	CaF_2 + apatite	CaF_2 + little apatite
48	3.3	CaF_2	CaF_2 + little apatite
1	3.0	CaF_2 + apatite	CaF_2 + apatite
24	3.0	CaF_2	CaF_2 + little apatite
48	3.0	CaF_2	CaF_2 + little apatite
1	2.0	CaF_2 + little apatite	CaF_2 + little apatite
24	2.0	CaF_2	CaF_2 + little apatite
48	2.0	CaF_2	CaF_2 + little apatite

The studies on powdered dental enamel treated with sodium monofluorophosphate did not disclose any change of apatite structure to one of calcium fluoride, regardless of the conditions employed. Treatments were made at pH values of 6.7, 4, 3, and 2, at times ranging from one hour to one week, with 2 per cent sodium monofluorophosphate solution. All electron diffraction and all x-ray diffraction patterns were of apatite alone. Therefore, it may be concluded that, under the conditions of these experiments, treatments with sodium monofluorophosphate do not result in any observable transformation of the apatite structure to a structure of calcium fluoride but that, rather, the apatite persists throughout the treatments. It is interesting to note that other workers[4] have pointed out the failure of this fluoride compound to reduce experimentally produced dental caries in the rat, as well as its failure to exhibit any effectiveness in reducing powdered enamel solubility in weak organic acids.

Treatments with stannous fluoride result in structural alterations which are decidedly different from those brought about by any of the other fluoride solutions so far investigated. The electron diffraction patterns of treated enamel indicate a predominately amorphous structure, while the x-ray patterns indicate a persistence of the apatite structure. Similar observations were made on all samples treated with 2 per cent stannous fluoride solution. The pH at the time of treatment ranged from 4 in some cases to as low as 1 in others, and the times of treatment varied from five minutes to one month.

Treatments with less concentrated stannous fluoride solution revealed similar results except in very short treatments in which the apatite structure persisted to some extent in the electron diffraction patterns. These results indicate that this reagent causes a transformation of the apatite structure to some sort of an amorphous structure, and that this change develops very predominately upon the surface of the enamel particles. It is not possible to ascertain from the results thus far whether the surface of the apatite is actually changed to an amorphous material or whether an amorphous substance is deposited upon that surface.

Efforts were made to render this surface material crystalline. It was found that the surface does become crystalline upon heating. The newly formed substance is neither apatite nor calcium fluoride, but its final identity has not yet been established fully. It is noteworthy, however, that the simple requirement that the reagent contain fluoride is not sufficient to provide for the formation of calcium fluoride.

In an effort to ascertain the relative roles of the tin and of the fluoride in these results, a series of experiments was conducted with stannous chloride solution. Portions of powdered dental enamel were treated with stannous chloride solution at pH values of 2.7 (unadjusted), 2, and 1, and at times of one hour, twenty-four hours, and one week. Electron diffraction patterns indicated that the apatite structure persists except in the most severe treatment (pH of 1 and time of one week), in which case the pattern indicated an amorphous structure. X-ray diffraction patterns were made of some of these samples, and they also revealed a persistence of the apatite structure except in the one most severe treatment, in which case the x-ray pattern also revealed an amorphous material. Comparison of these data with those of the stannous fluoride treatments discussed previously reveals that the two reagents behave very differently. The difference may be one of magnitude, since stannous fluoride resulted in amorphous electron diffraction patterns in five minutes, while stannous chloride caused an amorphous structure after one week at a pH of 1. However, the difference in magnitude is so great that it must be considered more as a difference in kind. Thus, both the tin and the fluoride must play essential roles in the action of stannous fluoride upon powdered dental enamel.

SUMMARY

Electron diffraction and x-ray diffraction studies have been made of powdered dental enamel following treatments with several different fluoride reagents. Potassium fluoride causes a transformation of the apatite structure to one of calcium fluoride, the change is predominately upon the surface of the enamel, and the rate of transformation increases as the pH is lowered. Sodium monofluorophosphate does not result in a change of apatite structure to calcium fluoride structure during treatments for as long as one week in a 2 per cent solution at a pH as low as 2. Sodium silicofluoride also causes a transformation to calcium fluoride, but the rate is slower than that with an alkali fluoride, and the change develops somewhat within the particles rather

than just on the surfaces. The effects of stannous fluoride are distinctively different from those of other reagents. The surface apatite structure is replaced by an amorphous structure, even during very brief treatments, and this amorphous substance may be rendered crystalline by heating. The mechanism must involve both the tin and the fluoride, because stannous chloride treatments result in practically no alteration of the original apatite structure.

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THE RELATIONSHIP OF TITRATABLE ACIDITY, TITRATABLE ALKALINITY, AND pH TO THE INCIDENCE OF DENTAL CARIES

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IN THIS paper we deal with three chemical characteristics of saliva, namely, titratable acidity, titratable alkalinity, and hydrogen ion concentration, and their relationship to dental caries incidence. A number of studies have been reported in which pH and extent of dental caries are considered. Most of these studies indicate a lack of close relationship between pH of whole saliva and dental caries of extent.

Fewer studies have been reported in which titratable acidity or titratable alkalinity and dental caries occurrence were the points of consideration. In these studies there is some uniformity in reports reflecting an association between dental caries and titratable alkalinity. Röse[1] (1905), Marshall[2] (1915), Hubbell[3] (1938), Dreizen, Mann, Cline, and Spies[4] (1946), and Sullivan and Storvick[5] (1950) reported an association between buffer capacity or titratable alkalinity and proneness to dental caries.

SUBJECTS AND METHODS

The 315 subjects for this series were a random sampling of greater Boston's school population as they were brought to the Forsyth Dental Infirmary for dental examination and care; the age range was 5 to 11 years. The number of children falling in either extreme of a statistical distribution of caries prevalance was small. Ten children had no fillings and no carious lesions detectable by explorer or x-ray examination. Thirteen children had more than thirty-one decayed or filled surfaces.

Three milliliters each of whole resting saliva was collected from each child. A minimum of two hours had elapsed since the last meal or other foods. The saliva was collected without physical or chemical stimulation other than the request to gather saliva in the mouth and place it in a given test tube. The saliva was immediately divided into 1 ml. portions (foam discarded), one each for the three tests under consideration: titratable acidity, titratable alkalinity, and pH.

Acidity was measured against 0.001 normal NaOH with phenolphthalein as the indicator; alkalinity was measured against 0.001 normal HCl with methyl red as the indicator; pH was determined with a Beckman potentiometer (laboratory model G), using calomel and glass electrodes.

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RESULTS

Figs. 1, 2, and 3 present the frequency distribution of these three characteristics for the same 315 children. Mean, standard deviation, mode, range, and a sampling of variance are carried with these figures. Variance is, of course, of primary importance. Therefore, the saliva of twenty-three children who came by chance from the admitting room to us was tested a second time. The

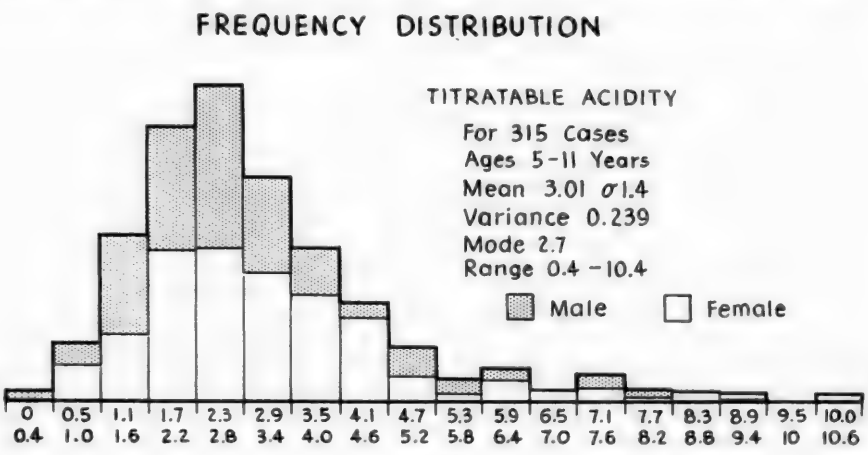

Fig. 1.

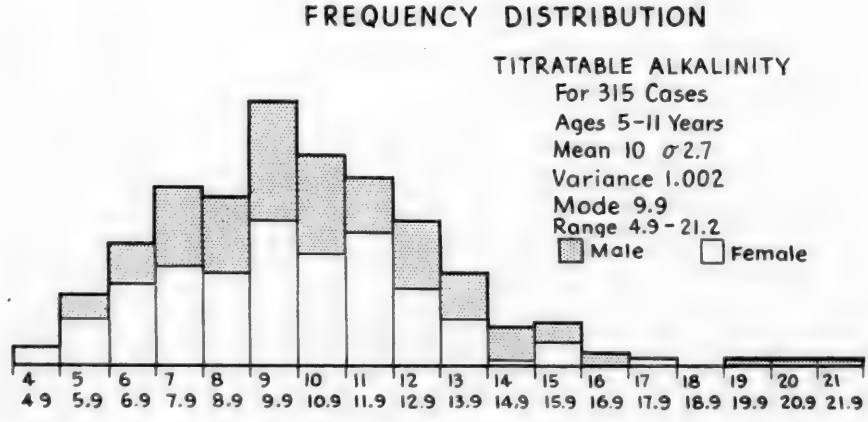

Fig. 2.

analysis of variance was according to the formula $\frac{d^2}{2N}$. Inspection of the figures will reveal that variance is from approximately one-third to one-sixth of one standard deviation. To ascertain whether the results of a longer series of retests would yield comparable data, two young adults were retested morning and afternoon twice a week for a four-week period. An analysis of variance

indicated variance for alkalinity to be 1.07, for acidity to be 0.315, and for pH to be 0.197. It would, then, appear that we are dealing with a reasonably reduplicable factor rather than a chance variable.

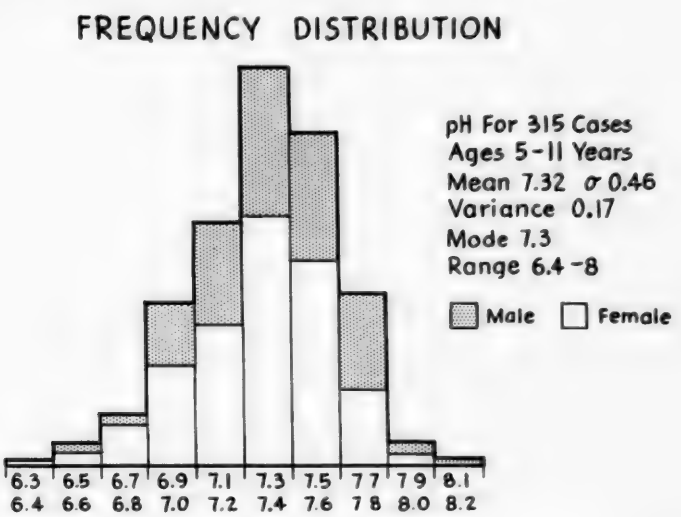

Fig. 3.

In attempting to determine the extent of decay we broke the data down in three ways.

1. D F (decayed and filled surfaces) $\begin{matrix}0\\1\text{-}30\\31+\end{matrix}$

2. D Q $\dfrac{\text{Sum of decayed (superscript)} + \text{filled (superscript)}}{\text{Tooth surfaces present}}$ $\begin{matrix}0\\0.01\text{-}0.5\\0.51\text{-}1.01\end{matrix}$

3. Classes of teeth involved:
   - Caries-free
   - Molars and premolars only involved
   - Canines and incisors also involved

TABLE I. FISCHER'S t VALUES SHOWING RELATIONSHIP OF D F TO pH, TITRATABLE ACIDITY, AND TITRATABLE ALKALINITY

TEST	CARIES EXTENT (D F SURFACES)	t	PROBABILITY THAT RELATIONSHIP IS NOT REAL
pH	1-30: 31+	0.375	>0.50
pH	0: 1-30	1.33	<0.10
pH	0: 31+	2.61	<0.02*
Titratable acidity	1-30: 31+	0.41	>0.50
Titratable acidity	0: 1-30	1.79	<0.10
Titratable acidity	0: 31+	2.52	<0.02*
Titratable alkalinity	1-30: 31+	1.74	<0.10
Titratable alkalinity	0: 1-30	3.65	<0.01†
Titratable alkalinity	0: 31+	3.25	<0.01†

*Possibly significant value.
†Significant value.

TABLE II. FISCHER'S t VALUES SHOWING RELATIONSHIP OF DENTAL QUOTIENT TO pH, TITRATABLE ACIDITY, AND TITRATABLE ALKALINITY

TEST	DENTAL QUOTIENT	t	PROBABILITY THAT RELATIONSHIP IS NOT REAL
pH	0.01-0.5:0.51-1.01	1.50	>0.10
pH	0:0.01-0.5	2.59	>0.01*
pH	0:0.51-1.01	2.46	=0.02*
Titratable acidity	0.01-0.5:0.51-1.01	1.43	>0.10
Titratable acidity	0:0.01-0.5	1.94	>0.05
Titratable acidity	0:0.51-1.01	2.13	<0.05
Titratable alkalinity	0.01-0.5:0.51-1.01	1.32	>0.10
Titratable alkalinity	0:0.01-0.5	2.98	<0.01†
Titratable alkalinity	0:0.51-1.01	3.22	<0.01†

*Possibly significant value.
†Significant value.

TABLE III. FISCHER'S t VALUES SHOWING RELATIONSHIP OF CARIES IN TEETH GROUPED ACCORDING TO CLASSES TO pH, TITRATABLE ACIDITY, AND TITRATABLE ALKALINITY

TEST	VARIATIONS IN CARIES INCIDENCE ACCORDING TO TOOTH CLASSES	t	PROBABILITY RELATIONSHIP IS NOT REAL
pH	Caries in canines and incisors vs. caries in molars and premolars only	0.71	<0.50
pH	Caries in molars and premolars vs. caries-free	1.32	>0.10
pH	Caries in canines and incisors vs. caries-free	3.96	<0.01*
Titratable acidity	Caries in canines and incisors vs. caries in molars and premolars only	0.16	>0.50
Titratable acidity	Caries in molars and premolars vs. caries-free	1.85	>0.05
Titratable acidity	Caries in canines and incisors vs. caries-free	1.87	>0.05
Titratable alkalinity	Caries in canines and incisors vs. caries in molars and premolars only	2.93	<0.01*
Titratable alkalinity	Caries in molars and premolars vs. caries-free	3.45	<0.01*
Titratable alkalinity	Caries in canines and incisors vs. caries-free	4.13	<0.01*

*Significant values.

TABLE IV. FISCHER'S t VALUES SHOWING RELATIONSHIP OF CARIES INCIDENCE TO CHEMICAL FACTORS WHEN EXTREME CASES ONLY ARE CONSIDERED (CARIES-FREE VERSUS HIGH CARIES INCIDENCE)

TEST	CARIES EXTENT	t	PROBABILITY THAT RELATIONSHIP IS NOT REAL
pH	Caries-free: 31+	2.61	<0.02
pH	0:0.51-1.01	2.46	=0.02
pH	Caries-free vs. caries in canines and incisors	3.96	<0.01
Titratable acidity	Caries-free: 31+	2.52	<0.02
Titratable acidity	0:0.51-1.01	2.13	<0.05
Titratable acidity	Caries-free vs. caries in canines and incisors	1.87	>0.05
Titratable alkalinity	Caries-free: 31+	3.25	<0.01
Titratable alkalinity	0:0.51-1.01	3.22	<0.01
Titratable alkalinity	Caries-free vs. caries in canines and incisors	4.13	<0.01

The Fischer t test was applied to test significance of relationship. Tables I, II, and III give the results for D F, D Q, and classes of teeth according to association with pH, titratable acidity, and titratable alkalinity.

Table IV summarizes the results of Tables I, II, and III when only the extreme cases are considered. Fig. 4 indicates graphically the association of titratable acidity, titratable alkalinity, and dental caries occurrence. There is an indication that low titratable acidity and high titratable alkaliniy tend to be found in the saliva of persons free from dental decay, while high titratable acidity and low titratable alkalinity tend to be found in the saliva of persons with very high dental caries experience. Moreover, it would appear from these studies that the relation of reserve basic, or alkaline, materials in the saliva is more closely and consistently related to dental caries than is either titratable acidity or pH.

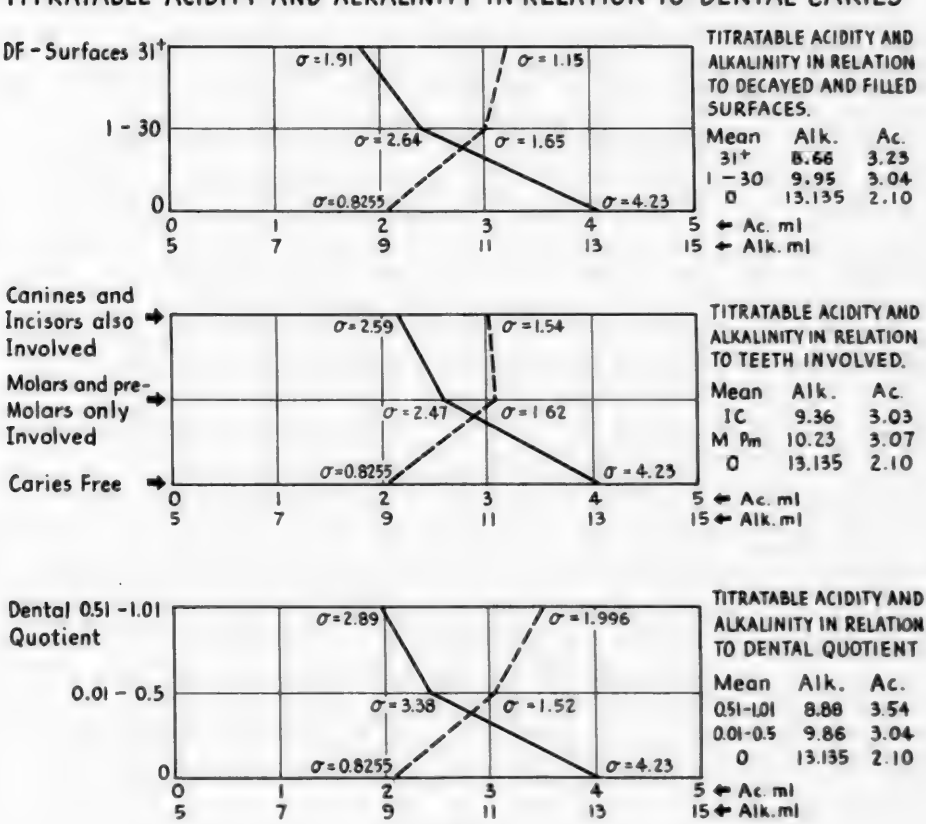
TITRATABLE ACIDITY AND ALKALINITY IN RELATION TO DENTAL CARIES
DF - Surfaces 31+
1 - 30
0
σ = 1.91
σ = 1.15
σ = 2.64
σ = 1.65
σ = 0.8255
σ = 4.23
0 1 2 3 4 5 ← Ac. ml
5 7 9 11 13 15 ← Alk. ml
TITRATABLE ACIDITY AND ALKALINITY IN RELATION TO DECAYED AND FILLED SURFACES.
Mean Alk. Ac.
31+ 8.66 3.23
1 − 30 9.95 3.04
0 13.135 2.10
Canines and Incisors also Involved
Molars and pre-Molars only Involved
Caries Free
σ = 2.59
σ = 1.54
σ = 2.47
σ = 1.62
σ = 0.8255
σ = 4.23
0 1 2 3 4 5 ← Ac. ml
5 7 9 11 13 15 ← Alk. ml
TITRATABLE ACIDITY AND ALKALINITY IN RELATION TO TEETH INVOLVED.
Mean Alk. Ac.
IC 9.36 3.03
M Pm 10.23 3.07
0 13.135 2.10
Dental 0.51 -1.01 Quotient
0.01 - 0.5
0
σ = 2.89
σ = 1.996
σ = 3.38
σ = 1.52
σ = 0.8255
σ = 4.23
0 1 2 3 4 5 ← Ac. ml
5 7 9 11 13 15 ← Alk. ml
TITRATABLE ACIDITY AND ALKALINITY IN RELATION TO DENTAL QUOTIENT
Mean Alk. Ac.
0.51-1.01 8.88 3.54
0.01-0.5 9.86 3.04
0 13.135 2.10

Fig. 4.

DISCUSSION

We should, perhaps, pause here to define our terms. First it should be made clear that in determining pH, we are dealing with hydrogen ion concentration, while in obtaining titratable acidity and titratable alkalinity we are

measuring potential production and conjugation of hydrogen ion. One example will illustrate the vast difference between pH and the titratable factor. The total or titratable acidities of 0.1 normal HCl and 0.1 normal acetic acid are the same despite the fact that the number of H^+ in the HCl solution is seventy times greater than in the acetic because of the almost complete dissociation of HCl and the low dissociation of acetic acid. In other words, the hydrogen ion of CH_3COOH in the titration method can be drawn off by addition of NaOH or other source of base.

For a common ground of understanding we will define an acid according to Brönsted's theory as any substance which gives off protons (H^+), and a base as any substance which combines with protons. The strength of bases depends on their ready combination with protons and the strength of the valence bond formed. Thus, an acid dissociates into a proton and a base, whereas a base combines with a proton to form an acid.[6]

It is of interest that the figures for alkalinities are frequently two or three times the values for acidities. This is true not only in our own studies but also in those reported by Marshall[2] and by Sullivan and Storvick.[7] It is a difficult point to interpret. One thinks of CO_2 loss. It may also be helpful to consider those units often accounting for acidity and alkalinity in body fluids.

The acid ions of most concern to the biochemist are H_3O^+ and NH_4^+; the bases of usual concern are OH^-, NH_3, and the ions of many weak acids, such as the carbonate ion (HCO_3^-), the acetic ion (CH_3COO^-), the phosphate ion ($H_2PO_4^-$), and so forth.[6] The amphoteric nature of amino acids and thus the amphoteric nature of peptide chains and proteins are well established. We should, therefore, look not only to the inorganic electrolytes but also to the protein constituents of saliva for explanations of the acidity, alkalinity, and caries-proneness relationships. It may be of some interest to direct our thinking to those proteins containing the strongly basic amino acids, namely, lysine and arginine.

It may be that these and certain other amino acids play a unique role in the oral enzyme systems that are protective for tooth structure. Sherman and Caldwell[8] reported that histidine and tryptophane have a unique inhibitory effect on the amyloclastic phase of starch digestion. Florestano, Faber, and James,[9] Turner and Crane,[10] and Sullivan and Storvick[5] have reported that high activity of the amyloclastic enzyme is associated with proneness to dental decay. Sullivan and Storvick further reported a correlation between buffer capacity and starch hydrolyzing time. Thus one's thinking is directed toward the effect upon amylase and other oral enzymes of the higher alkalinity of the saliva of persons free from dental decay.

It may also be that acidity-alkalinity relationships in the saliva may affect mineral exchange in the tooth. Possibly, if one has enough basic reserves, acidity can be handled without disturbing ionic exchange between saliva and tooth structure to the detriment of the latter. Rapp's[11] studies on calculus formation have raised the question of whether a moderate amount of alkalinization of saliva could cause sufficient oversaturation of calcium phosphate salts to facilitate precipitate formation.

The studies of Hodge and Leung[12] on calculus formation suggest some very interesting interrelationships in supersaturated versus unsaturated saliva.

SUMMARY AND CONCLUSIONS

The salivas of 315 children, aged 5 to 11 years, chosen at random from a school population brought for dental examination and care to the Forsyth Dental Infirmary were tested for titratable acidity, titratable alkalinity, and pH. The mean, mode, range, and standard deviation for each factor have been calculated.

When the children were divided into groups according to number of carious lesions, it was found upon application of the t test for significance of relationship that titratable alkalinity was consistently associated with the incidence of caries when caries-free persons were contrasted with those with extensive caries; titratable acidity and pH were less consistently associated with caries occurrence than was titratable alkalinity. There was a tendency for low acidity and high alkalinity of the saliva to be associated with caries-free persons, while high acidity and low alkalinity tended to be found in the saliva of children with a great amount of caries.

It is concluded that: (1) Reserve alkalinity is more closely and consistently related to caries experience than is either titratable acidity or pH. It would appear that the saliva of persons free from dental decay has a high potentially basic conjugate or an enzyme system capable of yielding this effect.

(2) When groups were divided on the basis of gradually increasing extent of caries, there was too much overlap to make these tests conclusive in terms of diagnosis and prognosis for the individual child. But this and other similar studies are definitive enough to direct our thoughts to a discriminative study designed to determine the various sources of basic conjugates in the saliva.

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THE AMINO ACID COMPOSITION OF PROTEINS ISOLATED FROM THE HEALTHY ENAMEL AND DENTIN OF CARIOUS TEETH

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THE organic matter in the healthy portion of carious teeth has not been subjected to as much study as have been the inorganic components. In general, it might be stated that no significant differences were found in the total organic matter from healthy and carious enamel estimated from nitrogen determination.[1, 2] It might be noted, however, that the variation in nitrogen content found by these investigators is great. Murray and Bowes[1] found 0.156 per cent nitrogen in the enamel of sound teeth, and Deakins and Volker[2] reported 0.28 per cent. Whereas Murray and Bowes found 0.157 per cent N in carious enamel, Deakins and Volker reported 0.22 per cent, and stated that differences between the normal and carious enamel were not significant in spite of the fact that they averaged 30 per cent. Since only average values were published, no statistical estimation of significance is possible. No studies on the nature of the isolated proteins from the two types of enamel have been found.

Hess, Lee, and Neidig[3, 4] have determined the amino acid content of the proteins isolated from the enamel and dentin of healthy human molars. In the present communication are given the results of the amino acid analyses of similar proteins isolated from the healthy portions of carious human molars.

EXPERIMENTAL

The enamel and dentinal proteins were prepared by the method of Losee, Leopold, and Hess.[5] The principal concern was to make certain that the enamel and dentin from the carious human molars were free from any carious material. The carious portion of the enamel was removed by scraping, and any stained enamel was removed by grinding with steel burs. The final criterion was that the enamel should show no discoloration when viewed under a dissecting microscope. In the preparation of the dentin, a similar technic was used, and all the discolored dentin was discarded. Losses of material were taken in order to ensure the removal of carious structure. The proteins were isolated from the enamel, and dentin was separated from five molars, altogether, seven such isolations were carried out, yielding seven samples of enamel and dentin proteins from thirty-five teeth. The samples were analyzed by the same methods used for the determination of the amino acids in the healthy teeth proteins.[3, 4]

These studies were substantially aided by a contract between the Office of Naval Research, Department of the Navy, and Georgetown University [Nonr 1049(00)] and also by a grant from the Sugar Research Foundation.

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Table I presents the data obtained on the proteins. The values are the averages of the determinations on the seven preparations. For comparison, the values previously obtained on the proteins isolated from healthy molars are included. The amino acid content is calculated on a 16.00 per cent nitrogen basis to facilitate comparison between the two sets of protein; the actual average nitrogen content with its standard deviation is also given. For the proteins isolated from the healthy portion of carious enamel, the amino acids accounted for 97.4 per cent of the nitrogen of the protein, and for the dentin protein, the recovery was 100.3 per cent.

TABLE I. AMINO ACID COMPOSITION OF PROTEINS ISOLATED FROM CARIOUS AND NONCARIOUS HUMAN MOLARS

	ENAMEL			DENTIN		
	CARIOUS*			CARIOUS†		
	AMINO ACID (PER CENT)	AMINO ACID NITROGEN (PER CENT)	NONCARIOUS AMINO ACID (PER CENT)	AMINO ACID (PER CENT)	AMINO ACID NITROGEN (PER CENT)	NONCARIOUS AMINO ACID (PER CENT)
Lysine	5.20	0.997	4.41	3.31	0.634	3.34
Arginine	8.15	2.621	7.91	7.18	2.301	7.40
Histidine	1.59	0.431	1.35	0.75	0.203	0.80
Phenylalanine	6.65	0.564	7.29	1.87	0.159	1.90
Methionine	2.66	0.250	1.18	0.50	0.047	0.50
Cystine	0.49	0.057	0.70	0.18	0.021	0.10
Tryptophan	0.79	0.108	0.12	0.00	0.000	0.00
Serine	6.23	0.830	7.65	3.14	0.419	3.50
Threonine	3.95	0.465	5.15	3.41	0.401	3.10
Glycine	21.10	3.937	20.74	22.22	4.146	22.50
Alanine	6.65	1.046	9.55	8.64	1.358	8.50
Tyrosine	0.41	0.032	1.22	1.29	0.100	1.10
Proline	7.14	0.869	6.39	16.03	1.950	16.25
Hydroxyproline	9.35	0.999	7.19	12.63	1.349	13.10
Aspartic acid	5.30	0.558	5.88	5.49	0.578	5.63
Glutamic acid	9.87	0.940	11.02	10.58	1.007	10.62
Valine	3.12	0.373	3.82	2.89	0.346	3.00
Leucine	2.81	0.300	5.29	3.86	0.312	3.60
Isoleucine	1.92	0.205	2.06	1.27	0.136	1.25
Hydroxylysine	0.00	0.000	0.000	1.00	0.184	1.00
Total		15.582			15.651	

*Calculated to 16.00 per cent N, determined N 15.80 ± 0.69 per cent.
†Calculated to 16.00 per cent N, determined N 15.80 ± 0.37 per cent.

DISCUSSION

It is apparent that the protein isolated from the healthy portion of carious dentin is identical in composition with that isolated from normal dentin. Atkinson and Matthews[6] found free aspartic acid and glutamic acid in carious dentin; this may well be possible, since the decomposition of the dentin could lead to the liberation of many free amino acids. These investigators also reported the presence of tryptophan in the dentin; this we are unable to confirm. No positive color test for tryptophan was given by the intact protein, using either the Hopkins-Cole or the p-dimethylaminobenzaldehyde tests. It is generally accepted that collagen does not contain tryptophan, and it would be rather unusual to find it in dentinal collagen.[7]

The protein isolated from the healthy enamel of carious teeth is chemically quite different from the protein isolated from healthy enamel of noncarious

teeth. This is rather surprising, but we feel that the data support the statement. The results were obtained from thirty-five teeth grouped so that seven separate protein samples were isolated. Each sample was analyzed, and the averages were presented. The individual differences between the various analyses were all within the limits of error of the methods. We feel reasonably sure that all the carious enamel was removed and that only healthy enamel was used. The histidine:lysine:arginine ratio for the carious enamel protein is 1:4:5, the same as for the normal enamel protein. The content of glycine, the highest of all the amino acids, is also the same for both proteins. Apparently, the basic protein structure is the same for both types, but there has been a marked alteration with respect to many amino acids, particularly methionine, tryptophan, threonine, alanine, tyrosine, leucine, and hydroxyproline.

We have no explanation to offer for these findings. We do not know whether the differences found preceded the caries or were initiated by it. We do not believe that the results are due to the inclusion in our samples of carious enamel and consequent contamination with carious enamel protein. The results do suggest a study of the proteins of animal teeth before and after the induction of caries and also during the preinduction period.

SUMMARY

The proteins were isolated from the healthy portions of carious human molars. Analyses of the amino acid content were performed on seven different preparations of the proteins, five molars being used for each preparation. The results were averaged, and the amino acid composition of the protein from the healthy portion of the carious dentin was practically identical to that of the protein obtained from normal dentin. The amino acid composition of the enamel protein isolated from the healthy portion of carious teeth showed marked differences from that of the protein isolated from normal enamel.

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LONGITUDINAL DATA ON ERUPTION AND ATTACK OF THE PERMANENT TEETH

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INTRODUCTION

TABLES on eruption of the permanent teeth were published as early as 1837, when Saunders counted the individual teeth present in 1,046 children of 9 and 13 years. He pointed out that the data were useful as a "test of age" to aid enforcement of an early act regulating child labor in England. Twenty years later, Cartwright counted the teeth in 3,074 children throughout the school-age range, and published a table which has served as a model for scores of additional investigations during the past century. We need review the findings only briefly, since many reviews are available (Bean, 1914; Suk, 1919; Wuorinen, 1926; Cattell, 1928; Klein, Palmer, and Kramer, 1937; Steggerda and Hill, 1942; Hurme, 1948; and Gödény, 1951).

Eruption is known to occur substantially earlier in girls than in boys, the average sex difference varying from about two months for upper first molars to nearly one year for lower canines. In some race groups, eruption occurs significantly earlier than in others, although race differences in respect to the sequence of eruption of individual teeth have not been found. The eruption of the first six pairs of permanent teeth (upper and lower first molars and first and second incisors) is usually followed by an interval when no teeth are erupting, so that the teeth tend to appear in two "batches." Some authors have reported small differences between right and left sides of the jaws, but since the differences have not been consistent from one study to another it is safe to assume that they are slight, if they exist at all. The sequence of eruption is known to differ in the maxilla and mandible, and certain sex differences have been found in the sequence, particularly in regard to the canines.

Dental caries attack, likewise, has been studied and reported by many people. A comprehensive review of this literature has been published (Finn, 1952). While caries attack displays race, sex, and geographic differences, in general the number of attacked teeth follows a consistent curve that rises during childhood, the teens, and early adult life, flattens out somewhat in approaching 35 years, but continues to rise slowly as long as teeth are present.

Practically all of the studies establishing our knowledge of eruption and attack have been "cross-sectional" in nature; that is, the conclusions were drawn from observations on different individuals in successive age groups. Several authors have noted that "longitudinal" or serial records kept on growing children might yield additional information, and a number of school

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systems and clinics have begun the collection of such data. Stones, Lawton, Bransby, and Hartley (1951) have reported on 329 children followed for three years.

The 100 children whose eruption and attack patterns are reported upon herein were followed for nine years or longer. They are a sample of the children on whom the Board of Education of Cleveland has kept dental records as long as the children remained in the Board's jurisdiction. Each child was examined about once each year. The condition in each of his twenty-eight tooth spaces at each examination was recorded on a card (herein called the "record card") designed by H. H. C. Wilson.

The completed record card thus shows the sequence of eruption, attack, and treatment in each tooth space of the child's mouth. The data are the more consistent and valuable because the examining was done by a limited number of dentists, who kept the records in conformity with a code printed on the card.

The Cleveland authorities kindly loaned us the completed record cards of children who were no longer being followed. For this report* we have selected the records of fifty boys and fifty girls, and have studied the data they contain on the permanent teeth. We have derived the form of the age curves of eruption and attack, the sequence of the median ages of eruption of the tooth pairs, the proportion of first molars among all attacked teeth, and the mortality rates of treated and untreated first molars.

We believe that the findings on the 100 children are instructive, and also that the methods we have used are worth reporting in some detail. The analysis of long-term serial records presents special problems, and a description of our procedures may be useful in connection with analyses of longitudinal records being accumulated elsewhere.

Except in regard to race,† the basis for selecting the 100 children was simply the age span over which they had been examined. We did not include a child unless his record showed he had been followed at least nine years; in most cases the first examination was before age 6 years and the last was after age 14 years.

In considering the findings of this report, it should be remembered that the number of cases is small and their representativeness is uncertain. Longitudinal methods have no merit for ensuring representativeness of sampling. The children whose records we have used might have been somewhat earlier or later maturing than average. However, an age curve derived from longitudinal records may be relied upon to bring out the nature and form of that curve with much more certainty than cross-sectional data.

*In an earlier report the same records were used together with data on Illinois children of earlier ages in a study of caries experience in the primary teeth (Fulton, 1952).

†The 100 cases were limited as far as possible to white children, since previous investigators have found small average differences in dental development between white and Negro groups. The Cleveland record cards do not state the child's race group. However, they give the school or schools which each child attended, and at our request the Cleveland authorities were able to classify the schools as "predominantly white," "mixed," or "predominantly Negro." Nearly 90 per cent of the children had attended the "predominantly white" schools, which is about what would be expected on the basis of census data. The 100 cases were drawn from the records of the children who had attended the "predominantly white" schools.

Since the methods we used to work up the Cleveland data are most easily understood in the light of the findings, the discussion of the methods will be postponed until the results are presented.

RESULTS

The results for the boys and girls are reported separately in all tables. The sex differences are in line with previous studies showing somewhat earlier ages of eruption and attack for the girls. In charting the main findings, however, it has not seemed essential to show the results for both sexes in more than one of the illustrations (Fig. 3), because the general pattern of eruption and attack is very similar for both groups.

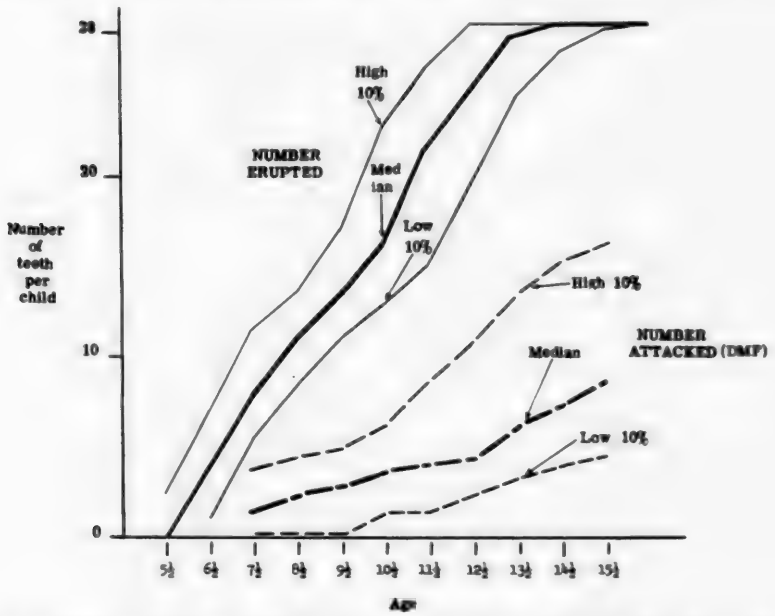

Fig. 1.—Number of teeth erupted and attacked at each age (data for fifty boys; see Table I).

As a measure of central or average tendency we have used the median, or the value above or below which lie one-half the cases. "High 10 per cent" and "low 10 per cent" points (described in detail later) are used to indicate individual differences. The spread between the high and low 10 per cent points for any given age represents the range of values found among the middle four-fifths (80 per cent) of the children; one-tenth of the children had values above the high 10 per cent point, and the values for another tenth were less than the low 10 per cent point.*

*As an alternative measure of individual differences, the "interquartile range" or difference between the upper and lower 25 per cent points has been used frequently in other studies. Except for giving a relatively narrow representation of individual differences, however, the interquartile range has no advantages. It is less reliable statistically, and it is slightly harder to find, than the difference between the high and low 10 per cent points. The latter measure is often called "D." If desired for comparison with other studies, interquartile ranges may be estimated by taking 53 per cent of the D values shown in this report; similarly, standard deviations may be estimated by taking 39 per cent of the D values. The standard error of D is approximately 0.9 D/ $\sqrt{N}$ (Kelley, 1924, pp. 104-105).

Age data are shown for 5½ years, 6½ years, and so forth, in order to facilitate comparison with the data of other studies, which are usually in terms of "age at last birthday"; grouping in the latter terms means, of course, that the values center on the half years.

Age Curves.—The data in Fig. 1 confirm the existence of a moderate bend or "dip" in the age curve for number of teeth erupted, and some tendency toward a "plateau" in the curve for number of teeth attacked.

Probably owing to chance, the plateau in number of attacked teeth is not as marked for boys as for girls (Table I). As would be expected, for both sexes the plateau in the number of attacked teeth lags one or two years behind the dip in the curve for number of teeth erupted.

TABLE I. NUMBER OF TEETH ERUPTED* AND NUMBER OF TEETH ATTACKED† (DMF)

AGE (YR.)	FIFTY BOYS			FIFTY GIRLS		
	LOW 10%	MEDIAN	HIGH 10%	LOW 10%	MEDIAN	HIGH 10%
			Erupted Teeth			
5½	0	0	2.2	0	0	4.8
6½	1.0	3.7	6.6	1.0	6.0	9.2
7½	5.2	7.8	11.2	4.6	9.2	11.9
8½	8.2	10.8	13.2	8.6	12.0	15.4
9½	10.6	13.1	16.6	11.3	14.0	21.0
10½	12.5	15.9	22.4	12.0	19.4	25.2
11½	14.6	21.0	25.6	14.4	24.3	27.4
12½	19.2	24.4	28.0	20.0	26.6	28.0
13½	23.8	27.3	28.0	24.0	28.0	28.0
14½	26.4	28.0	28.0	26.9	28.0	28.0
15½	27.6	28.0	28.0	28.0	28.0	28.0
16½	28.0	28.0	28.0	28.0	28.0	28.0
			Attacked Teeth			
5½	0	0	0.2	0	0	1.0
6½	0	0.1	1.7	0	1.0	2.7
7½	0	1.1	3.4	0	1.6	3.6
8½	0	2.0	4.0	0	2.9	4.0
9½	0	2.4	4.5	0	3.3	4.2
10½	1.0	3.2	5.7	0	4.0	5.7
11½	1.0	3.6	8.2	0.4	4.0	8.0
12½	2.0	4.0	10.3	1.4	5.1	11.3
13½	2.9	5.6	13.0	3.6	6.6	13.6
14½	3.6	6.7	14.7	5.1	8.7	17.1
15½	4.0	8.1	15.7	5.4	10.4	17.9
16½	–	–	–	6.3	11.0	19.3

*Medians represent average number of erupted teeth per child. Low and high 10 per cent points represent individual differences, that is, the range of values for the middle 80 per cent of the children at each age.

†Medians represent average number of DMF teeth per child. Low and high 10 per cent points represent individual differences, that is, the range of values for the middle 80 per cent of the children at each age.

These departures from straight-line development were studied by examining the individual curves of eruption and attack for each child. In some of the individual curves the departures were much more conspicuous than in others. The departures were matters of degree, and there were no distinct "types," but the facts may be described approximately as follows:

In about one fifth of the children of either sex, the individual curves showed approximately straight-line increases up to age 12 or 13 years, in

respect to both number of teeth erupted and number attacked. In about three fifths of the children, moderate departures from straight-line development appeared. This group included a few cases where one could suspect that a marked departure occurred, but if so, it was greatest at a particular age when the child was not examined. In the remaining fifth of the children, it was evident that marked departures from straight-line development had occurred in respect to both eruption and attack.

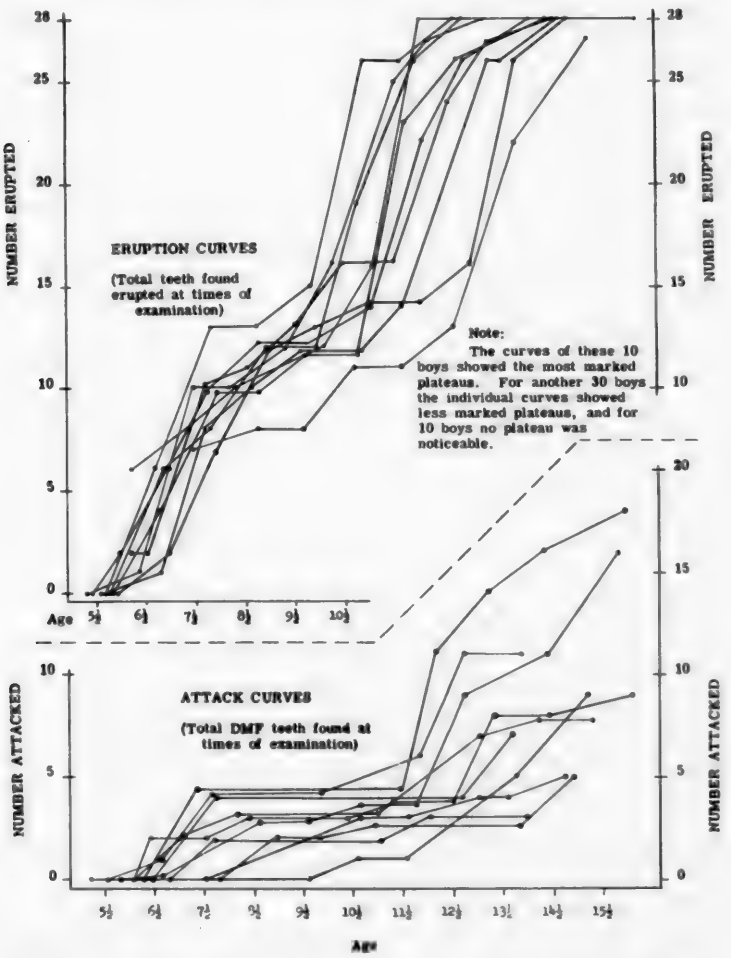
NUMBER ERUPTED
ERUPTION CURVES
(Total teeth found erupted at times of examination)
Note:
The curves of these 10 boys showed the most marked plateaus. For another 30 boys the individual curves showed less marked plateaus, and for 10 boys no plateau was noticeable.
NUMBER ATTACKED
ATTACK CURVES
(Total DMF teeth found at times of examination)
Age

Fig. 2.—Individual curves of eruption and attack for ten selected boys.

The curves for the ten boys showing the most marked departures are presented in Fig. 2. Similar curves were found for the ten most extreme cases among the girls; if there was any sex difference at all, the girls seemed to show the departures more strikingly than the boys. We stress that the

cases shown in Fig. 2 are not typical of the majority of children, but present the curves to show their reality in some children.

A point that may have some practical significance for the majority of children, however, is the fact that the percentage of attacked permanent teeth found in the mouth is relatively high at ages 9 or 10 years; at ages 11 or 12 years it becomes somewhat lower, and it then rises rapidly during adolescence. The age curve for this percentage (see medians in Table II) has the same general form in both sexes, although the rise and fall in the boys' curve are at slightly older ages than the corresponding points in the girls' curve. The method of deriving the percentage values will be described later. The results shown in Table II are merely a further reflection of the fact that some degree of departure from the straight-line development occurs in the majority of children.

Eruption Sequence.—We did not attempt to estimate the eruption age for each of the twenty-eight teeth separately, but rather for each of the fourteen

TABLE II. PERCENTAGE OF ERUPTED TEETH THAT WERE ATTACKED*

AGE (YR.)	FIFTY BOYS			FIFTY GIRLS		
	LOW 10%	MEDIAN	HIGH 10%	LOW 10%	MEDIAN	HIGH LOW %
5½	†	†	†	†	†	†
6½	†	†	†	†	†	†
7½	0.0	16.3	34.7	0.0	18.7	34.1
8½	0.0	18.8	32.6	0.0	22.6	30.8
9½	0.0	20.1	30.1	0.0	21.6	27.0
10½	7.2	20.0	29.0	0.0	20.1	27.8
11½	7.4	17.1	33.5	1.4	18.5	31.1
12½	10.4	17.7	38.4	7.0	20.6	41.7
13½	11.8	21.7	46.9	14.2	24.8	51.0
14½	12.8	24.5	51.2	19.0	31.5	61.3
15½	13.7	28.0	57.5	19.9	37.3	63.9
16½	-	-	-	23.5	40.9	68.7

*Values for medians and ranges are analogous to those in Table I.
†Not computed because number of erupted teeth too small.

TABLE III. ERUPTION AGES OF TOOTH PAIRS*

	100 BOYS			100 GIRLS		
	LOW 10%	MEDIAN	HIGH 10%	LOW 10%	MEDIAN	HIGH 10%
Upper teeth						
No. 1	76.5	87.0	100.5	72.0	84.0	99.0
No. 2	87.0	101.0	115.0	82.5	94.5	108.0
No. 3	119.5	136.5	155.0	107.5	126.5	149.0
No. 4	105.0	125.0	144.5	101.0	119.0	141.0
No. 5	117.0	133.5	156.0	107.0	125.5	149.5
No. 6	70.0	79.5	90.0	66.5	76.5	89.0
No. 7	128.5	145.0	165.5	123.5	139.0	160.0
Lower teeth						
No. 1	69.5	78.5	89.0	65.0	74.5	85.0
No. 2	80.0	90.5	103.0	72.0	83.0	97.0
No. 3	113.0	129.0	144.0	97.0	116.0	138.5
No. 4	114.5	133.0	155.0	102.0	121.0	140.5
No. 5	123.0	141.5	162.5	113.0	129.5	157.0
No. 6	67.5	76.5	89.0	64.0	72.5	82.0
No. 7	124.5	142.0	160.0	113.5	131.0	154.0

*Figures are ages in months. Values for medians and ranges are analogous to those in previous tables.

pairs of teeth. It was apparent, in view of individual differences, that more records should be used for this part of the work. Accordingly, the records of an additional fifty boys and fifty girls were scored in respect to eruption age of each tooth pair.

The results for the 100 boys and 100 girls are shown in Fig. 3. The findings are by no means new, but we believe our arrangement of the data has some advantage over the arrangements used in earlier reports, particularly for bringing out the reasons for the departures from straight-line development in Figs. 1 and 2.

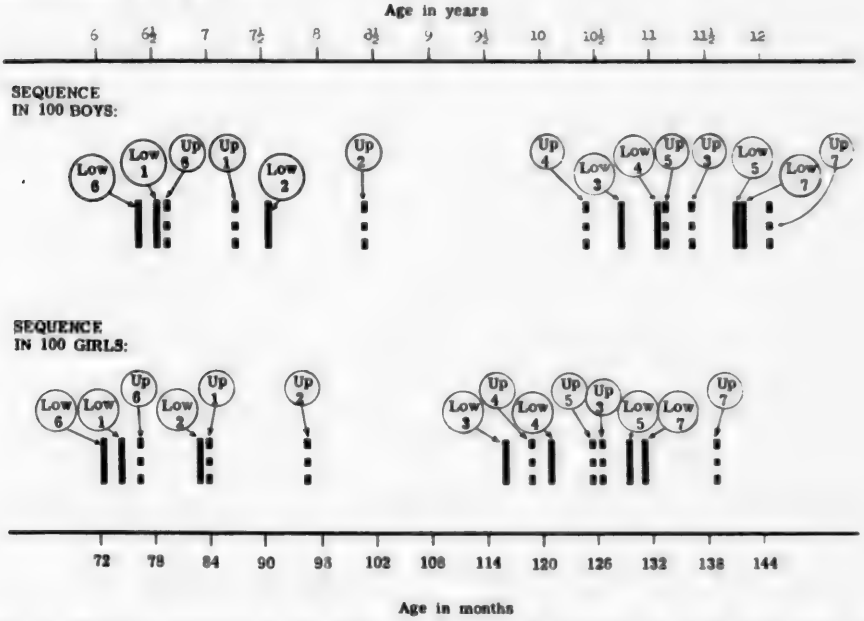

Fig. 3.—Eruption sequence of the teeth (median age of eruption for each tooth pair; see Table III).

While the grouping or batching of eruption times holds true for the statistically "average" child, individual differences in eruption age for any tooth pair are very great (see high and low 10 per cent values in Table III). In consequence of this variation, one would expect the eruption of the fourteen pairs of teeth to be spaced out more or less evenly over the age range 6 to 12 years in some children, while in others the grouping of eruption times would be even more striking than in Fig. 3. Inspection of individual records has shown that both kinds of cases occur, although the most frequent and typical cases are like those in Fig. 3. In view of the small numbers of cases we have used, however, reliance should not be placed on the exact sequence of eruption or on the sex differences in that sequence suggested by our data.*

*The average ages published by Hurme (1949) are probably the most reliable because they are compiled from twenty-four studies representing over 93,000 children of European descent. The two batchings of the teeth are more distinct in our data. This may mean that our children were a relatively homogenous group. In part, the difference may also reflect the fact that we used median ages and Hurme used mean ages, for, as Hurme pointed out, the distributions of later erupting teeth are skewed toward the older ages. This necessarily makes median ages somewhat lower than mean ages for late erupting teeth.

Attack Rate in First Molars.—In the light of earlier evidence regarding the relatively high susceptibility of the first molars or No. 6 teeth, it seemed worth while to estimate the proportion of total DMF accounted for by attacked No. 6's at particular ages. Inspection of the records indicated that the pertinent facts could be brought out by examining the data on DMF at ages 10½ and 13½ years. Accordingly, the records of the initial group of fifty boys and fifty girls were analyzed for DMF components at those ages.

The results are shown in Fig. 4 and Table IV. In the boys at age 10½ years, the total number of attacked teeth (DMF including attacked No. 6's) averaged 3.5 teeth per child. Among these teeth, the 6's comprised 3.0 teeth, or 86 per cent of DMF teeth at age 10½ years.

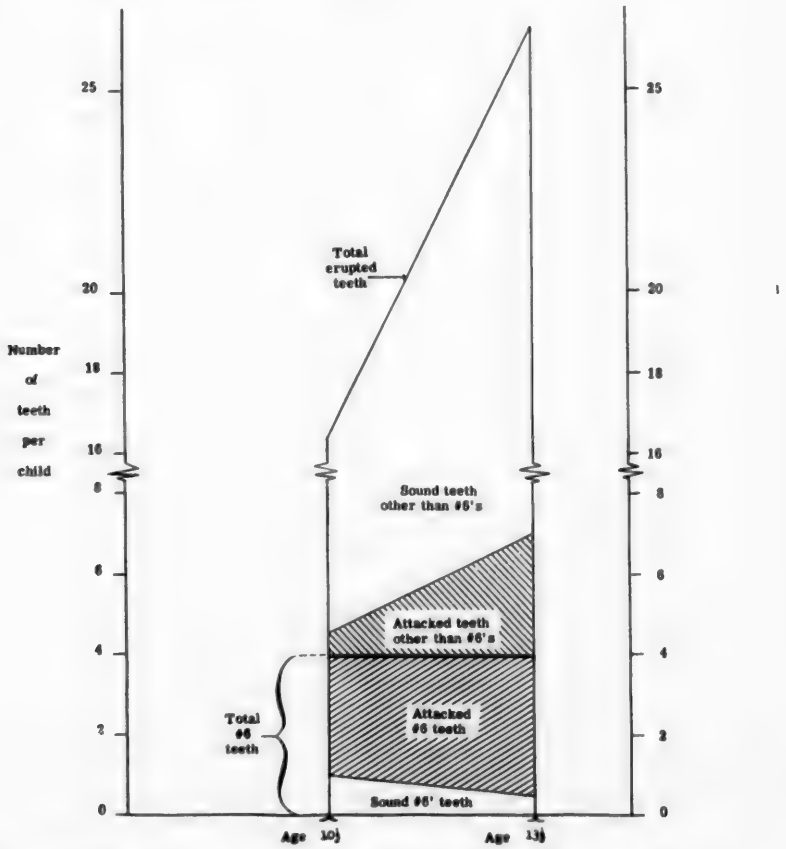

Fig. 4.—Amount of attack in No. 6 teeth (averages for fifty boys; see Table IV).

In the girls at the same age, slightly more teeth were attacked (3.7 per child), but the number of attacked No. 6 teeth was greater also (3.2). Thus among all attacked teeth, the No. 6's comprised 87 per cent, or about the same for girls as for boys.

TABLE IV. DMF PER CHILD IN FIRST MOLARS AND OTHER TEETH

	FIFTY BOYS		FIFTY GIRLS	
	AGE 10½	AGE 13½	AGE 10½	AGE 13½
No. 6 teeth				
D	1.7	1.4	1.6	0.6
M	0.6	1.3	0.9	1.7
F	0.7	0.8	0.7	1.2
Sound	1.0	0.5	0.8	0.5
	4.0	4.0	4.0	4.0
Other teeth				
D	0.1	1.8	0.4	2.3
M	0.3	0.2	0.0	0.3
F	0.1	1.0	0.1	1.7
Sound	11.8	19.7	14.3	18.7
	12.3	22.7	14.8	23.0
Total erupted teeth	16.3	26.7	18.8	27.0
Total DMF	3.5	6.5	3.7	7.8
D	1.8	3.2	2.0	2.9
M	0.9	1.5	0.9	2.0
F	0.8	1.8	0.8	2.9

D: attacked and not filled or filled and subsequently attacked.
M: missing or indicated for extraction.
F: filled and not further attacked.

By age 13½ years, of course, a relatively large number of teeth other than the No. 6's were attacked. Yet the No. 6 teeth had continued to suffer attack, with the result that in both sexes an average of only 0.5 of all four first molars were sound at age 13½ years.

One cannot assume that either the total DMF rate or the absolute sizes of the D, M, or F components shown in Table IV are representative of children in general. Yet the proportionate amount of attack shown in Fig. 4 for the No. 6 teeth, as compared with the other teeth, may be considered typical in view of the similar findings of Cohen (1936) and Knutson, Klein, and Palmer (1938).

These results bring into sharp relief an already known fact—the desirability of examining the first molars for evidence of attack soon after their eruption. Indeed, it would not seem essential, at least in a limited program, to examine any of the permanent teeth except the first molars among children under age 10 years.

Effect of Treatment.—A problem regarding which serial data are of unique interest and value is the extent to which fillings can save or lengthen the life of attacked teeth.

Of all 400 first molars in the 100 children, we found that 37 of these teeth had suffered no attack during the period the children were followed, and 97 had not been attacked until after age 10 years. For studying the effects of treatment we thus had 266 first molars which had been attacked and followed long enough to yield evidence on the question of effects of treatment.

Among the 266 attacked teeth, 121 had been treated and 145 had not. A comparison of the mortality rates of the two latter groups of teeth measures the effectiveness of treatment under ordinary conditions of practice.

Of the 121 attacked teeth which had received treatment, 21 teeth (17 per cent) were lost despite the treatment. Their loss did not seem to be caused by belated treatment, since the average interval between attack and treatment was no longer for these teeth than for the 100 teeth which were treated and saved. It thus appeared that most of the 21 teeth lost despite treatment were unusually susceptible to caries attack, although it is possible that a few of them could have been saved with closer or more continuous attention.

Of the 145 attacked teeth which did not receive treatment, 128 teeth (88 per cent) were lost. The other 17 teeth were followed for periods of five to seven and one-half years after the attack was first noted, and were recorded as carious (but not indicated for extraction or missing) over those periods.

If the 17 attacked teeth which survived without treatment could have been followed longer, however, most of them would probably have been recorded as lost in a few more years. This may be inferred from the data on the 128 teeth known to have been lost. Of them, 59 were lost within two years after attack; 42 were lost in two and one-half to four years; 24 were lost in four and one-half to six years; and 3 were lost in six and one-half to eight years. It thus appears that if the 17 teeth not known to have become lost had been followed longer, they would probably have extended the distribution to twelve or perhaps fourteen years, but few, if any, would be expected to survive for longer periods.

METHODS

Each child's record card showed his date of birth, the dates when he was examined, and the condition found in each tooth space at each examination. Our first problem was to design a form on which this information could be represented in terms of the child's ages, in months, when he was examined.*

The form is an eight and one-half by fourteen inch mimeographed sheet used lengthwise. The essential part of the form is twenty-eight horizontal rows of dots. Each row is labeled to represent one of the twenty-eight tooth spaces in the mouth. These rows are grouped in four sets of seven rows each, representing the four mouth quadrants. Each of the rows is 132 dots (eleven inches) long. At intervals along the margins of the sheet, the dots are labeled to represent months of age from 60 to 192 months, or 5 to 16 years.

Converting Dates to Ages.—A separate "calendar scale" was made from a long strip of heavy paper. The same typewriter was used in preparing this scale as in preparing the mimeographed form, so that the calendar months of time on the scale were exactly comparable, unit for unit, with months of age on the form. Along the top margin of the calendar scale the letters JFMAMJJASOND were typed sixteen times in succession. The letters represented the calendar months within one year; the successive cycles of letters represented years and were labeled 1932, 1933, and so forth, to 1947.

*A copy of the form which we used for that purpose will be furnished by the senior author on request. However, investigators wishing to study cases covering different age ranges could easily prepare similar forms suited to their own purposes from the description of our form.

With such a scale it was easy to convert the dates of examination from a child's record card directly into months of age on the mimeographed form.

For example, suppose a child's record card showed he was born in January, 1930, and was first examined in April, 1935. For this child, as for all others, five years were added mentally to the date of birth, because the beginning point on the mimeographed form was age 5 years. The point on the calendar scale representing this date (January, 1935, in the given case) was then set opposite age 5 years or 60 months at the bottom of the mimeographed form. The scale was held in this position while the dates of all examinations were located on the scale, and converted "automatically," as it were, into the child's ages on the form. The first examination in the given case was in April, 1935; the point on the form corresponding to this point on the scale showed that the child was 63 months old at the time, and a mark was made on the form at that point. Similarly, with the calendar scale in the same position, the next examination date was located along the calendar scale, translated into the child's age by marking the form at the corresponding point, and so on for later examinations.

Starting with the points so marked at the bottom of the form, we drew light lead-pencil lines up through the rows of dots. Thus there were as many of these lines as there were examinations of the child; their locations on the form showed the child's ages when examined, as well as the intervals of age between examinations, and the total age range over which the child was followed.

Transcribing Dentists' Entries.—We then used a colored pencil to copy onto the form the coded entries which had been made on the child's record card for the various tooth spaces at successive examinations. These entries were copied over the light lead-pencil lines already made to represent the examination ages. Green and red pencils were used to distinguish between the entries for deciduous and permanent teeth, although this was not essential. When completed, these entries gave an accurate and complete picture, by age, of all the changes that had been observed in the child's mouth.

This method yields the necessary information with a minimum of clerical work, and we believe the procedure is less susceptible to clerical errors than other methods of arranging detailed information from longitudinal records.

For some types of study, it might be desirable to copy all entries from the dentists' original records for all examinations. However, we found that time was saved without loss of accuracy by copying all entries for the first examination only; for subsequent examinations we did not copy the entry for a tooth space unless it showed some change from the condition recorded for the same tooth space in the previous examination.

After the information from a child's record card had been transcribed to the mimeographed form in this manner, the margins of the form were used for scoring the number and kind of teeth erupted, the number and kind

attacked, and whether the latter were D, M, or F teeth. These counts were placed in the margins opposite the respective examination ages, as shown by the lead-pencil lines.

Analysis of Age Curves.—Our general method of analyzing the age data is best described by citing, as an example, the procedure used to derive the three lines in the upper part of Fig. 1, which show the number of teeth erupted among the fifty boys as they passed from age 5 to age 15 years.

It will be noted, first, that the values in the chart are for regular intervals of age, that is, ages 5½, 6½, and so forth. The original values for each child were not in such terms, of course, because the examinations were distributed irregularly throughout calendar years. There was no tendency for the examination to cluster around certain ages. Although the average interval between examinations was about twelve months, some intervals were less than six months and a few were as long as two years.

To obtain values in terms of regular intervals of age, all fifty of the boys' individual age curves for number of erupted teeth were plotted on one large chart. On this chart the plotting was carried out for whatever months of age each boy actually was when examined, and the values for his successive examination ages were connected. This yielded a composite chart of fifty curves for number of erupted teeth.*

On the whole, the tendency of the fifty curves to run parallel courses was striking. This tendency was greater than might be judged from the cases shown in Fig. 2, which are selected for their departures from straight-line development. There appeared to be no reason for singling out groups of earlier-maturing or later-maturing children for separate analysis.

To find the median number of erupted teeth at age 8½ years, for example, we drew a vertical line through the fifty curves with a colored pencil, starting up from the base line of the composite chart at the point representing 102 months (8½ years) of age. By counting the individual curves which intersected the colored line, we located the points where that line was crossed by the twenty-fifth and twenty-sixth individual curves. These points were often the same because of concentration of cases around the median. When that was the case, the point where both twenty-fifth and twenty-sixth curves crossed the colored line was taken as the median, but where they crossed the colored line at different points, the mid-point between the intersections was used.

To obtain the high 10 per cent point, we started from the top of the chart and found the point where the fifth and sixth individual curves crossed the colored line representing age 8½ years. The mid-point between their intersections was used as the high 10 per cent value. The low 10 per cent point was located similarly, except that counting for the fifth and sixth lines was started at the bottom of the chart.

*Provided the plotting is done carefully on a large-scale chart, we believe a usable composite could be made for as many as seventy-five individual curves. With more than that number of cases, it would be best to divide them randomly into two or more groups, plot the groups separately, and average the results from the separate composites.

To obtain the lower set of curves in Fig. 1, we plotted on another large chart the individual curves of the fifty boys for number of teeth attacked. Methods analogous to those described previously were used to derive the medians and the high and low 10 per cent values for number of attacked teeth at each age.

To obtain the values for the fifty girls, their individual curves for eruption and attack were plotted on separate charts, and the same methods were used. The results are included in Table I.

To derive the data in Table II, or the percentage of erupted teeth that were DMF at each age, two different procedures were possible. Since neither procedure was necessarily ideal and since each appeared to have merit, we decided to use both methods and to average the values so obtained. As the first procedure, we computed all values in Table I, Attacked Teeth data, as percentages of the corresponding values in Table I, Erupted Teeth data. This was done for all high and low 10 per cent values, as well as for the medians at the various ages. The second procedure was analogous, except that we began with the data for each child. His erupted and attacked teeth at each examination age had been totaled on the margins of his mimeographed form. The percentage of erupted teeth which were attacked was computed on the form for each of the child's examination ages. The percentage values for the fifty boys were then plotted on one large chart, and the percentage values for the fifty girls were plotted on another. Using the graphic method already described, we located the medians and high and low 10 per cent points for each sex at each year of age.

The values obtained by the two procedures were then averaged to obtain the figures in Table II. As already noted, we believe some significance attaches to the trend of the medians in either sex, but it should be added that this trend emerges only as an average effect in sizable groups of children. We have not charted the data in Table II because the composites prepared in the second procedure described previously showed that the individual percentage curves did not follow parallel courses to a marked extent, and a chart with lines connecting the values in Table II would represent few individual children.

Other Statistical Methods.—To study the eruption ages of the fourteen pairs of teeth, we located on each child's mimeographed form the last examination age when a given tooth pair had not appeared and the first examination age when it was recorded as present. The mid-point of this interval, that is, the average of the two ages, was easily read from the form, and was used as the most probable eruption age of the tooth pair. Occasionally the examinations indicated that the teeth of a pair had erupted at different times; we then found the mid-point of the previously mentioned interval for each tooth separately and used the average of these mid-points as the eruption age of the pair.

From these values we cast up, for the boys, the distribution of their 100 eruption ages for the tooth pair No. 1, the distribution for tooth pair No. 2,

and so forth, and likewise for the girls. From these distributions we obtained the medians and high and low 10 per cent points shown in Table III for the fourteen tooth pairs.*

To obtain the data for Fig. 4 and Table IV, we noted on each child's form the examination ages which, for him, were closest to ages 10½ and 13½ years. A coin was flipped to decide which examination to use in the rare case when one was given six months before and another six months after age 10½ or 13½ years. The D, M, F, and sound teeth were then totaled separately for first molars and other teeth at each of the selected examination ages. For each sex group, division of these totals by fifty gave the figures in Table IV.

To study the effects of treatment, the record for each of the 400 first molars was copied from the mimeographed sheets onto a separate slip of graph paper. Ages were read and copied only to the nearest one-half year because, for the purpose concerned, more precise age data would have had little significance. It was then easy to try out a number of different groupings and subgroupings of the slips. The groupings already described appeared to be the most meaningful classification for the amount and kind of data we had at hand. With longitudinal records over longer periods of time, however, it might be worth while to study in more detail the effects of age at eruption, interval between eruption and attack, and interval between attack and treatment.

SUMMARY

1. Methods for analyzing longitudinal records of dental conditions are described.

2. Application of the methods to serial data on 100 Cleveland school children confirms the existence of considerable departures from straight-line development in the age curves for total teeth erupted and total teeth attacked. Although no such departures were found in the curves of about one fifth of the children, in three fifths of the cases there were moderate departures, and in the remaining fifth the departures were marked. The material suggests that age data on eruption and attack obtained from cross-sectional studies tend to mask the extent to which plateaus exist in individual children. Study of the median eruption ages of the fourteen tooth pairs confirms the belief that the reason for the plateaus is the marked batching of the teeth in respect to eruption age.

3. Of all attacked permanent teeth, about 86 per cent are accounted for by attacked first molars at age 10½ years.

*The records of a few girls did not begin early enough to show eruption ages of their lower No. 1's and No. 6's, and the records of a few boys did not continue long enough to show eruption of their No. 7 teeth. Yet the records of even these children gave some useful, if negative, information; they at least showed that the uncertain teeth were already erupted by the first examination age, or had not erupted by the last examination age.

Since the eruption ages of all tooth pairs could be estimated directly for over four-fifths of the children, it is not likely that the median value for any tooth pair was significantly affected by the information which was missing for extreme cases. However, in Table III the low 10 per cent points shown for early-erupting pairs and the high 10 per cent points shown for late-erupting pairs are relatively unreliable, because, in part, they had to be guessed by considering both the cases where direct estimation was possible and the cases where the records were incomplete.

4. Effectiveness of treatment has been studied by analyzing the data on 266 first molars attacked by age 10 years, among which 121 had been treated and 145 had not. Of the treated teeth, only 17 per cent were lost during the period covered by the records. Of the untreated teeth 88 per cent were lost, and the distribution of their survival times indicated that most of the remaining 12 per cent would have been reported lost a few years later if the children had been followed longer.

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HORMONAL CONTROL OF TOOTH ERUPTION

I. The Effect of Thyroidectomy on the Upper Rat Incisor and the Response to Growth Hormone, Thyroxin, or the Combination of Both

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INTRODUCTION

NORMAL eruption, from the viewpoints of both time and position of each individual tooth, is one of the decisive events in the development of a well-formed and balanced dentition. While all the changes in the intraosseous position of a developing tooth germ are classified as "pre-eruptive movements," "eruption" designates its movements from the pre-eruptive location in the jaw into the functional position in the oral cavity. A review of the literature reveals a great number of theories of the process of eruption, but only a small amount of factual evidence is available.[14]

In order to ascertain metric and histologic data concerning the hormonal control of tooth eruption, rats were subjected to various endocrine experiments. The findings will be presented in a series of three articles. The first is concerned with the effects of thyroidectomy at birth and subsequent substitution therapy with growth hormone or thyroxin or the combination of both.*

For some time clinical reports have indicated that a relationship between thyroid disturbances and tooth eruption may exist. However, a hormonal theory was not advanced before Keith[12] held the thyroid and pituitary glands responsible for the eruption process. The effects of thyroidectomy on dental structures were first studied by Erdheim in rats[7] and by Biedl in dogs.[5] They consisted for the main part in delayed eruption and defective formation of the tooth structures. Ziskin, Salmon, and Applebaum[20] from a study of rats, operated at birth and at 7 days of age, considered retardation of eruption time, dentin and root development, especially in the molar, as typical thyroprivic symptoms. Eruption remained delayed when thyroidectomized rats were chronically injected with parathormone; treatment with thyroid extract, on the other hand, effected partial restoration of tooth development, while the combination of both hormones produced complete recovery. Thyroidectomy performed in monkeys by Ziskin resulted in a decreased daily rate of dentin formation as revealed by alizarinization.[21]

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*The second article deals with the effects of hypophysectomy following progressively longer intervals; the third one reports on the response of the incisors of hypophysectomized rats to growth hormone and tnyroxin given separately or in combination.

Hyperthyroidism was produced experimentally in newborn rats by Hoskins[10] and by Karnofsky,[11] and caused precocious tooth eruption. Normal adult rats injected with thyroxin showed increased eruption rate of the incisors.[9]

In normal adult Norwegian rats, a 2.1 mm. weekly average eruption rate of the upper incisors has been reported.[1, 16] Schour[18] found a weekly average of 1.98 mm. in white rats of an unspecified age. In a more recent study[8] reference is made to 2.3 mm. average weekly eruption rate in 68- to 138-day-old white rats. Since measurements of the eruption rate and the dimensions of the incisors of thyroidectomized and subsequently treated rats have not been reported, such a study appeared of considerable basic and practical importance.

EXPERIMENTAL ARRANGEMENT

One normal control group and four experimental groups of 63-day-old male and female rats of the Long-Evans strain were used. The experimental rats were thyroidectomized at the day of birth; one group of those was retained as operated controls; another was injected with a daily dose of 0.5 mg. purified pituitary growth hormone intraperitoneally from their thirty-second to sixty-third day of life; the next group received 2.5 micrograms of dissolved thyroxin (Squibb) subcutaneously for thirty-two days; and the last group was given the same dosages of growth hormone and thyroxin in combination.

With the beginning of the thirty-second day, when three experimental groups received hormonal treatment, a marking was made weekly with a fine jeweler's file at the gingival margin of the labial surface of the upper incisor to study the eruption rate. The distance between two lines thus represented the rate of eruption for a seven-day period. Average measurements are computed in Table I.

TABLE I

WEEKLY ERUPTION RATE OF UPPER INCISORS BETWEEN THIRTY-SECOND AND SIXTY-THIRD DAYS OF LIFE

	GROUP	NUMBER OF RATS	TREATMENT* DAILY DOSE EXCEPT SUNDAY	WEEKLY RATE OF ERUPTION (MILLIMETERS)	WEEKLY RATE OF ERUPTION (PER CENT)
I	Normal	15	0	2.8 ±0.17	100
II	Thyroidectomized	4	0	1.56 ±0.57	55
III	Thyroidectomized + growth hormone	6	0.5 μg growth hormone	1.82 ±0.21	65
IV	Thyroidectomized + thyroxin	6	2.5 γ thyroxin (Squibb)	2.38 ±0.14	82
V	Thyroidectomized + combination	6	0.5 μg growth hormone 2.5 γ thyroxin	2.4 ±0.17	83

*Treatment was given from the thirty-second to the sixty-third day of life.

After the animals were sacrificed at their sixty-third day of life, the heads were split in half and roentgenographed. With a microcaliper the following measurements were taken from the roentgenograms.

(a) The thickness of the upper incisor at the alveolar crest.
(b) The radius of the outer curvature (labial surface) of the upper incisors*
(c) The mesiodistal length of the upper three molars.
(d) The space between the distal surface of the upper incisor and the mesial surface of the upper first molar
(e) The intermolar width between the lingual surfaces of the upper third molars

The group averages, with standard deviations, are seen in Table II. The cranial halves were then further dissected for sagittal histologic sections of the upper incisors; they were stained with hematoxylin-eosin.

TABLE II

MEAN VALUES OF DENTAL MEASUREMENTS IN MILLIMETERS

	GROUP	NUMBER OF RATS		A THICKNESS OF UPPER INCISOR	B RADIUS OF UPPER INCISOR	C LENGTH OF UPPER MOLARS	D LENGTH UPPER INCISOR – M_1	E WIDTH UPPER $M_3 - M_3$
I	Normal	11	M	2.52	5.93	8.0	11.6	8.51
			SD	±0.06	±0.11	±0	±0.17	±0.22
II	Thyroidectomized	10	M	2.08	4.78	6.90	8.23	8.46
			SD	±0.08	±0.23	±0.26	±0.37	±0.30
III	Thyroidectomy + Growth	8	M	2.03	4.9	6.92	9.40	9.06
			SD	±0.09	±0.11	±0.19	±0.32	±0.39
IV	Thyroidectomy + Thyroxin	12	M	2.30	5.49	6.98	9.82	8.32
			SD	±0.09	±0.23	±0.14	±0.31	±0.29
V	Thyroidectomy + Combination	12	M	2.32	5.59	6.9	10.2	8.95
			SD	±0.03	±0.19	±0.18	±0.33	±0.15

M = mean.
SD = standard deviation.

RESULTS

1. In normal young rats the weekly eruption rate of the upper incisors averages 2.8 mm. (Table I). Hence, it takes a continuously growing incisor approximately five weeks to erupt from the apex to the gingival margin.

In the histologic sections (Fig. 1) it can be seen that the enamel epithelium extends over the entire unerupted labial portion of the incisor and forms gingivally the epithelial attachment to the enamel (Fig. 10, *A*). Oral epithelium is not directly attached, but fuses for a short distance with the basal layer of the reduced enamel epithelium. At the apical region of the enamel organ, columnar inner enamel epithelium encompasses almost the entire cervical loop (Fig. 6). They originate from a center of proliferation at the outer basal side and border the interior cells of the loop which are differentiated into the stratum intermedium and stellate reticulum cells. The bony fundus (*F*) shows evidence of resorption at the inner aspect and apposition at the outer surface.

2. Thyroidectomy effects a drastic reduction of the eruption rate by 45 per cent, or a weekly average of 1.5 mm. (Table I). The tooth dimensions are decreased approximately 20 per cent (Table II, a, b, and c). In contrast to a

*When this circumference was not circular, the radius was determined from a center which was in equidistance to three points: the apex, the central portion, and the incisal end.

normal eruption period of five weeks, an interval of eight to nine weeks is necessary for the eruption from the apex to the gingival margin. This length of time is confirmed by the observation that the incisors of two thyroidectomized rats were without enamel at the incisal end so that oral epithelium was directly attached to dentin (Fig. 10, *B*). Thyroidectomy at birth therefore produced enamel aplasia which became clinically visible after sixty-three days. The dimensions of the upper jaw as measured in the roentgenograms (Table II) are more reduced in length than width; the distance between the incisor and first molar is 30 per cent shorter than normal; the intermolar width is decreased only 10 per cent.

Figs. 1-5.—Longitudinal sections of the upper incisors of rats, 63 days of age, representing the experimental Groups I to V. (Original magnification, ×11, reduced ½.)
Fig. 1.—Normal rat incisor. Specimen 9195, Plate 1976.
Fig. 2.—Thyroidectomized at birth. Specimen 9315, Plate 1968.

Histologically (Fig. 2), a marked reduction in vascularity of the pulp and hypercalcification of bone and dentin are conspicuous. Continuous dentinogenesis has narrowed the pulpal lumen; the lingual alveolar bone is sclerotic with a fibrous degeneration of the hemopoietic marrow. Modeling resorption is depressed and all tissues show a decreased amount of cellular elements. In the epithelia, proliferation is stunted and histodifferentiation retarded. The cervical loop of the enamel organ is displaced toward the interior of the pulp and consists of a cluster of epithelial cells of equal size

(Fig. 7). The characteristic components of the enamel organ at this point show little or no differentiation. The bony fundus lacks the processes of osteogenic transformation as seen in the normal controls. The enamel epithelium of the apical third produces a reduced amount of enamel, even though apparently normal in structure, and degenerates precociously in the incisal third so that oral epithelium, instead of the reduced enamel epithelium, maintains the gingival epithelial attachment.

3. Administration of growth hormone to thyroidectomized rats elicits a 10 per cent increase in the eruption rate to 1.8 mm. weekly. The radius of curvature slightly exceeds the untreated thyroidectomized controls (Table II), while the thickness of the incisor and the mesiodistal width of the molars remain the same. The jaw dimensions, however, are considerably increased and the intermolar width is even greater than normal average.

The resumption of growth is manifested histologically by the enrichment of the connective tissues by vascular and cellular elements as seen in the pulp, periodontal membrane, and lamina propria (Fig. 3). The periapical tissue is voluminous and interspersed with a great amount of tissue fluid. The dentinal walls at the apical third are long and slender, bordered by a dense layer of regular odontoblasts. The dentin is hypocalcified as calcospherites remain isolated. The bone shows a continuous lining of formative cells. The marrow spaces are larger and contain some hemopoietic elements. The epithelia have regenerated; the reduced enamel epithelium extends to the margin of the gingiva forming its epithelial attachment (Fig. 10, *C*). Proliferation of the cervical loop is reactivated at the outer basal side (Fig. 8), but the young cells remain small and fail to undergo histodifferentiation as seen in the normal. At the site more advanced in development, no signs of amelogenetic disturbances are noticeable.

4. Substitution therapy with thyroxin raises the eruption rate of the incisor 28 per cent to a weekly average of 2.38 mm. (Table I). The radius of curvature, though greater than in the growth hormone group, does not reach the normal range (Table II). The alveolar structures in reverse respond less to thyroxin than to growth hormone, as evidenced by an unchanged intermolar width.

The histologic analysis of their incisors reveals distinct differences from those of the growth hormone-treated rats (Fig. 4). The connective tissues are almost hyperemic; this is especially obvious in the pulp. The newly formed dentinal walls are comparatively less massive at the lingual side, although equally hypocalcified. The bone is porotic due to a rapid increment of the myelogenous component. The epithelial tissues are in a highly proliferative state. This is true for oral epithelium, as well as enamel epithelium at the cervical loop (Fig. 9). Enamel matrix formation proceeds regularly; enamel maturation, however, is disturbed due to early degeneration of the cells of the stratum intermedium. Hypoplastic enamel of various degree is the result (Fig. 10, *D*).

5. Administration of both growth hormone and thyroxin with a 2.4 mm. weekly eruption rate has practically the same effect as thyroxin given alone. Tooth and jaw measurements remain in the upper range of the thyroxin-treated group (Table II).

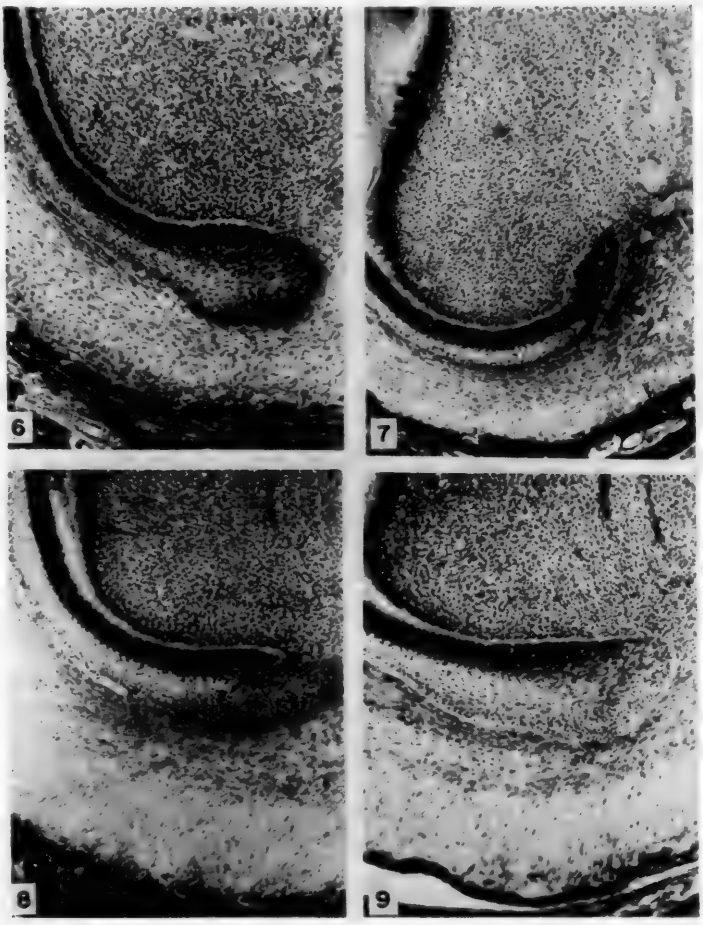
Histologically the dental and periodontal tissues nearly correspond to those of the normal controls, save for a somewhat greater vascularity and

Fig. 3.—Thyroidectomized at birth, from the thirty-second to the sixty-third day given daily growth hormone. Specimen 9252, Plate 1991.

Fig. 4.—Thyroidectomized at birth, from the thirty-second to the sixty-third day given daily thyroxin. Specimen 9342, Plate 1966.

Fig. 5.—Thyroidectomized at birth, from the thirty-second to the sixty-third day administered thyroxin and growth hormone. Specimen 9260, Plate 1710.

higher cellular activity of the connective tissues (Fig. 5). The enamel organ at the cervical loop is similar in appearance to that of the thyroxin-treated rats, while at the sites of enamel maturation it is comparable to the growth hormone-treated rats. The occurrence of enamel hypoplasia is not observed, but some delayed enamel maturation is found (Fig. 10, *E*).



Figs. 6-9.—The cervical loops of the enamel organ of the upper incisor of rats, 63 days of age, representing various experimental groups are seen. (Original magnification, ×110, reduced ½.)

Fig. 6.—In the normal rat inner enamel epithelium is differentiated at the basal periphery of the loop. Specimen 9336, Plate 1987.

Fig. 7.—In the thyroidectomized rats the loop shows a displaced position and lack of histodifferentiation. Specimen 9251, Plate 1985.

Fig. 8.—After growth administration to the thyroidectomized rat, the loop shows resumed proliferative activity at the basal periphery but no immediate histodifferentiation. Specimen 9252, Plate 1989.

Fig. 9.—On thyroid therapy in thyrodectomized rats, growth and histodifferentiation are restored to almost normal. Specimen 9342, Plate 1986.

DISCUSSION

With this first experiment, metric and histologic studies have disclosed that the eruption of the rat incisor is dependent upon the proper function of the thyroid. Two findings stand out:

1. Thyroidectomy does not completely arrest the eruption rate, while thyroxin substitution restores it to almost normal.
2. Growth hormone has little effect upon the eruption rate, while it considerably enhances alveolar growth.

These data reflect upon the eruption rate or the quantitative aspect, as well as upon the eruption mechanism or qualitative aspect.

Fig. 10.—The labial papillae of the upper incisor of rats of 63 days of age representing the various experimental groups are shown. (Original magnification, ×125, reduced 9/11.) *A*, The epithelial attachment of an intact rat is shown. Specimen 9636, Plate 1971. *B*, In the thyroidectomized rat, oral epithelium is attached to the dentin. Note hyperkeratinization of the gingiva. Specimen 9317, Plate 1969. *C*, Growth hormone elicits rejuvenation of the gingival tissues of the thyroidectomized rat. Specimen 9329, Plate 1791. *D*, Thyroxin therapy restores the gingival attachment of the reduced enamel epithelium and enhances proliferation of the oral epithelium. Note the presence of hypoplastic enamel. Specimen 9259, Plate 1970. *E*, Combination therapy has a beneficial effect upon all components of the gingival tissue. Specimen 9352, Plate 1992.

A. The Quantitative Aspect of Eruption.—The weekly eruption rate of 2.8 mm., found in these intact young rats of the Long-Evans strain, exceeds the data reported in the literature.[1, 8, 16, 18] This may be due to the comparatively younger age as well as the difference in strain of the rats studied.

Thyroidectomy reduces both eruption rate and size of the incisors, but in different ratio relative to normal average; the eruption rate is decelerated 45 per cent, while the size is decreased only 20 per cent. Concurrent studies indicate similar results in the molar region.[2] The third molars did not erupt, and the roots of the first and second molars were incompletely formed. Furthermore, the heights of the mandibular body and the ramus were reduced 25 per cent.[3]

Upon thyroxin therapy, the incisal dimensions increase comparatively less than the eruption rate. Eruption process and growth process obviously respond differently to the same hormone. Evaluation of the effects of growth hormone injections to thyroidectomized rats supports this concept. Although growth hormone brings about a greater increase in cranial structures than thyroxin, it has almost no quantitative effect upon eruption. Furthermore, the combined administration of both hormones elicits no greater weekly eruption rate than thyroxin given separately, while it engenders a slight increase in dental dimensions.

The moderate synergistic effect of combined therapy on dental growth is a sequela of a hypophyseal syndrome of thyroidectomy. The reduced output of gonadotropin and growth hormone of thyroidectomized rats was ascertained through the work of Scow, Simpson, Asling, Li, and Evans[17] and Becks, Scow, Simpson, Asling, Li, and Evans[4] in relation to somatic and skeletal changes and through the demonstration of the histologic changes in the pituitary by Koneff, Scow, Simpson, Li, and Evans.[13] More information regarding the interplay of growth hormone and thyroxin in the eruption process thus is to be expected from a study of the effects of the removal of the pituitary which presides over the production of both hormones.

B. The Qualitative Aspect of Eruption.—The thyroprivic condition of the incisor is characterized by a marked decrease in vascularity of the pulpal and periodontal tissues and by a stunted growth and retarded differentiation of the enamel organ at the cervical loop. Dentinogenesis and amelogenesis are depressed, but not completely arrested. Thyroxin treatment restores vascularization of the dental tissues and proliferation of the odontogenic epithelium. Dentinogenesis and especially amelogenesis became more disturbed in the phase of calcification, rather than during matrix formation. Enamel hypoplasias evidently have little bearing upon the eruption rate, which always is accelerated. The reverse is true for the effects of growth hormone injections. Amelogenesis and dentinogenesis are improved qualitatively, but eruption remains almost as tardy as in the thyroprivic state. It is concomitant with reduced vascular supply of the pulp tissue and depressed epithelial activity of the cervical loop. The essential factors determining eruption thus prove to be vascularization of the dental tissues and proliferation of the odontogenic epithelium.

The importance of vascularity in the eruption process has been recognized[6] for a long time and has been supported in recent years on the basis of overwhelming circumstantial evidence.[14, 19] The action of additional factors such as a "hammock ligament" was emphasized in order to explain the direction of the forces of eruption.

The significance of the growth activity of the odontogenic epithelial sheath with regard to tooth eruption, however, has found little consideration in the dental literature. Conjecture was made that the epithelial sheath occupies always a stable position[15] and that a proliferation at the free end would close the epithelial apical foramen.[19] Present observations revealed continu-

ous resorption at the inner aspect of the alveolar fundus of the incisor of normal, as well as treated thyroidectomized, rats. This indicates that positional changes of the odontogenic epithelial diaphragm take place. Only the fundus of thyroidectomized rats with decelerated tooth eruption showed aplasticity and stability. A firm fundus thus seems to be incompatible with the increase in size of the incisor. Furthermore, it is difficult to reconcile the contention that epithelial growth should be located between the free margin of the loop and the beginning of the predentin with various histologic facts. In accordance with the rule that highly specialized cells such as the inner enamel epithelium have lost the potency to proliferate, present observations give uniform evidence that the epithelial growth takes place at the outer basal side of the loop. From there the inner enamel epithelium cells travel around the loop to the inner side, while the new outer enamel epithelial cells move upward on the outer side of the enamel organ. Since differential growth rate of this odontogenic epithelium at the various sides of the diaphragm induces the growth movements of the tooth, this epithelial activity constitutes in fact the "steering mechanism" of the eruption process.

SUMMARY

For the study of the hormonal control of tooth eruption in this first of three endocrine experiments, sixty male and female rats of the Long-Evans strain were subjected to thyroidectomy at birth. One group of them served as operated controls and three groups received from the thirty-second to the sixty-third day of life, replacement therapy with growth hormone, thyroxin, or the combination of both. A group of intact 63-day-old rats was included as normal controls.

1. Thyroidectomy reduced the eruption rate 45 per cent, during a ten-week period, while the size of the tooth structures remained 20 to 25 per cent under normal average. Histologically, reduction of vascularity-stunted growth, as well as differentiation, and early atrophy of the odontogenic epithelium were conspicuous thyroprivic symptoms.

2. Administration of growth hormone elicited a slight increase in eruption rate (10 per cent) and in tooth size, while a considerable growth spurt of the maxillary bone over normal dimensions was observed. Histologically, connective tissues were rejuvenated, but young epithelial cells failed to undergo immediate histodifferentiation.

3. Injection of thyroxin increased the eruption rate (27 per cent) more than dental dimensions. There was a marked increase in vascularization of the dental structures and accelerated proliferation and histodifferentiation of epithelial tissues. Calcification, especially of the enamel matrix, however, was deficient.

4. When both growth hormone and thyroxin were injected simultaneously, the eruption rate and dental dimensions exceeded only slightly the thyroxin-treated group, but the histologic aspect revealed improved structures hardly to be distinguished from normal.

Appreciation is expressed to Dr. Robert O. Scow, who performed the skillful thyroidectomies on the rats used in this experiment, and to Dr. D. A. Collins for collecting some of the biometric data.

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HORMONAL CONTROL OF TOOTH ERUPTION

II. The Effects of Hypophysectomy on the Upper Rat Incisor Following Progressively Longer Intervals

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GROSS information on the influence of the pituitary gland on dental development emanated from clinical observations made particularly in acromegalics (P. Marie, 1886, Cushing, Keith). Subsequently, this endocrine gland was made responsible for abnormal tooth forms (Ascoli, Aschner, Kraus), diastema formation (Korkhaus, R. Schwarz), and abnormal tooth migration in paradentosis (Weinmann, Starke). The hazards of experimental hypophysectomy of earlier workers (Paulesco, 1891; Ascoli, Crowe, Cushing, and Morsley, 1900-1911) were greatly reduced through the introduction of the parapharyngeal approach to the hypophysis by Aschner,[1] in dogs, and Smith,[14] in rats. Extensive studies by Evans[7] and others first served to analyze the polyglandular effects of hypophysectomy. Concerted attempts resulted in the identification of at least six different hormones of the anterior lobe, three of which have been purified in recent years.

The effects of hypophysectomy on the rat incisors were first assessed histologically and roentgenographically by Schour and Van Dyke.[12] They studied 23 rats which were hypophysectomized at between 34 and 64 days of age. In a postoperative interval of 63 to 459 days, progressive retardation of eruption, arrested increase in the size, and distortion of the form of the incisor constituted their general findings. Histologically the enamel epithelium was found to undergo regressive changes, ultimately leading to cessation of amelogenesis while dentinogenesis proceeded continuously and obliterated the pulpal cavity. Arrest of eruption and continuous dentin formation at the basal end allegedly caused apical folding. Therefore, it was inferred that hypophysectomy produces a dissociation of the growth process from that of eruption.[11] Held,[8] in a study of the early changes of hypophysectomy in the rat incisors, could not find alterations in the calcification picture of the dentin, but claimed decreased resistance to infection of the periodontal structures. Becks, Collins, Simpson, and Evans,[4] in a study based on 142 hypophysectomized rats, including postoperative intervals between 6 and 640 days, concluded that a thickening of the dentinal walls at the expense of the pulp chamber was the only constant pathognomonic symptom of hypophysectomy. Distortion and folding of the

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TABLE I

AVERAGE DIMENSIONS OF THE UPPER INCISORS OF NORMAL AND HYPOPHYSECTOMIZED RATS AS MEASURED FROM ROENTGENOGRAMS (MILLIMETERS)

GROUP I. NORMAL CONTROLS																				
Age at autopsy (days)	25	28	31	35	36			45	56	68	84	105	116	135		253	307		449	629-659
Number of rats	3	1	8	3	1			1	6	6	7	3	4	4		4	2		2	5
Thickness	1.9	1.9	1.95	2.06	2.0			2.2	2.3	2.51	2.75	2.76	2.9	2.97		3.06	3.2		3.3	3.2
Curvature radius	4.9	4.9	4.95	5.06	5.0			5.1	5.9	6.1	6.4	6.8	6.9	6.85		7.06	7.3		7.3	7.4

GROUP II. HYPOPHYSECTOMIZED RATS																			
Postoperative interval (days)	0	6	8	10	12	14	16	28	43	56	77	87	100	132-147	279	332-347	450-563		
Age at autopsy (days)	28	34	36	38	40	42	44	56	71	84	105	115	128	160-175	307	360-375	478-491		
Number of rats		4	4	4	4	3	3	1	1	1	1	5	6	1	1	4	5		
Thickness of incisor		1.94	2.05	2.05	2.05	2.0	2.05	2.1	2.0	2.0	2.2	2.1	2.15	2.1	2.2	2.2	2.2		
Curvature radius		5.0	5.05	5.05	5.1	5.0	5.0	5.1	5.0	5.0	5.1	5.1	5.2	5.2	5.3	5.35	5.38		

apical tissues occurred inconsistently even among the hypophysectomized animals which survived very long postoperative intervals.

It is the purpose of this second experiment to ascertain correlated metric and histologic information of the effects of hypophysectomy, particularly on two phases of dental development, growth or increase in tooth size and eruption.

EXPERIMENTAL ARRANGEMENT

The material used in this experiment includes three groups of female rats of the Long-Evans strain.

Group I, consisting of 70 intact rats, represents the normal controls. Sixty of these rats were sacrificed after progressively longer intervals in order to obtain records of the size as compiled in Table I and the histology of the incisors at various ages. In the remaining 10 rats, the eruption rate was measured weekly for 51 weeks (357 days) beginning at 28 days of age.

Group II, consisting of 70 rats, were hypophysectomized at 28 days of age.* Sixty of them were sacrificed for metric and histologic purposes after progressively longer intervals, matching the ages of the normal controls (Table I). Nine operated rats of this group were kept alive for 51 postoperative weeks (357 days), during which the eruption rate was recorded (Table II). The average measurements of the radius of curvature and the weekly eruption rate of five, ten, and forty-seven postoperative weeks are computed in Table III with those of the normal controls of Group I.

TABLE II

AVERAGE ERUPTION RATE OF RATS HYPOPHYSECTOMIZED AT 28 DAYS OF AGE (MILLIMETERS)

Postoperative days	7	14	21	28	35	42	56	104	147	286	351
Postoperative weeks	1	2	3	4	5	6	8	11	21	40	51
Number of rats	9	9	9	9	9	9	9	6	6	5	4
Total extruding length	2.0	3.1	4.01	5.1	6.1	6.75	8.02	10.18	12.58	15.18	17.13
Weekly eruption rate	2.0	1.1	0.91	1.09	1.0	0.65	0.61	0.72	0.24	0.26	0.18

Group III is represented by 10 hypophysectomized rats which were sacrificed after long postoperative intervals (332 to 444 days). The eruption rate of the incisors was recorded during the last seven weeks of life. Individual tooth measurements at the day of autopsy, the total rate of eruption, and the incidence of apical folding, as judged from the histologic sections, are computed in Table IV.

The procedure for the measurements of the eruption rate was similar to that described in Paper I of this series[2] except that, in the old hypophysectomized animals, the markings had to be made in three- and four-week intervals because of the extremely slow eruption. At autopsy, lateral roentgenograms of the split cranial halves were measured with regard to the following dimensions of the first incisors: (1) thickness or mesiodistal diameter at the

*Criteria for judgment on completeness of operation as well as the diet on which the operated animals were maintained are described elsewhere.[5]

TABLE III

COMPARISON OF RADIUS OF CURVATURE AND WEEKLY ERUPTION RATE OF UPPER INCISORS IN NORMAL AND HYPOPHYSECTOMIZED RATS

GROUP	AGE AT OPERATION (DAYS)	RADIUS OF CURVATURE				WEEKLY ERUPTION RATE				
		NO.	AGE AT AUTOPSY (DAYS)	AVERAGE MM.	STANDARD DEVIATION	NO.	STUDY PERIOD (DAYS)	AVERAGE MM.	STANDARD DEVIATION	PER CENT
1A, Young normal	—	11	63	5.9	0.11	10	28-63	2.73	0.10	100
2A, Young hypophysectomized	28	6	63	5.05	0.19	9	28-63	1.21	0.15	45
1B, Adult normal	—	3	105	6.8	—	10	28-98	2.66	0.13	100
2B, Adult hypophysectomized	28	3	105	5.1	0.05	9	28-98	0.63	0.03	24
1C, Old normal	—	5	449	7.3	—	10	28-329	2.51	0.13	100
3, Old hypophysectomized	28	10	444	5.39	0.21	10	332-444	0.3	1.6	12
3A, Old unfolded	28	4	444	5.57	0.10	4	332-444	0.55	0.62	22
3B, Old folded	28	6	444	5.26	0.49	6	332-444	0.12	0.28	4

level of the alveolar crest, (2) radius of outer curvature. After fixation, the jaws were prepared for histologic analysis as specified.[2]

TABLE IV

THE RELATIONSHIP BETWEEN ERUPTION RATE, TOOTH DIMENSION, AND APICAL FOLDING IN 10 HYPOPHYSECTOMIZED RATS AFTER LONG POSTOPERATIVE INTERVALS

NO.	SPECIMEN	POST-OPERATIVE INTERVAL	AGE AT AUTOPSY (DAYS)	THICKNESS (MM.)	RADIUS (MM.)	ERUPTION IN 7 WKS. (MM.)	APICAL FOLDING
1	8762	332	359	2.1	5.6	3.4	0
2	8764	333	360	2.1	5.1	1.2	x
3	8763	340	367	2.0	5.5	0.7	x
4	8767	347	374	2.2	5.6	3.7	0
5	8766	387	414	2.1	5.2	0.9	x
6	8768	387	414	2.2	5.6	3.7	0
7	8765	389	416	2.2	5.5	4.8	0
8	8760	396	423	2.1	5.3	0.6	x
9	8761	402	429	2.2	5.2	0.7	x
10	8750	444	471	2.1	5.3	1.3	x

Legend: 0 = no folding.
x = apical folding.

FINDINGS

1. In plotting the measurements of the radius of curvature of the incisors of normal rats at various ages in the graph, Fig. 1, it is found that a period of rapid increase is terminated at 119 days. During the following thirty-four weeks, the radius increases continuously, but slowly, 0.5 mm. The thickness of the incisor behaves accordingly (Table I).

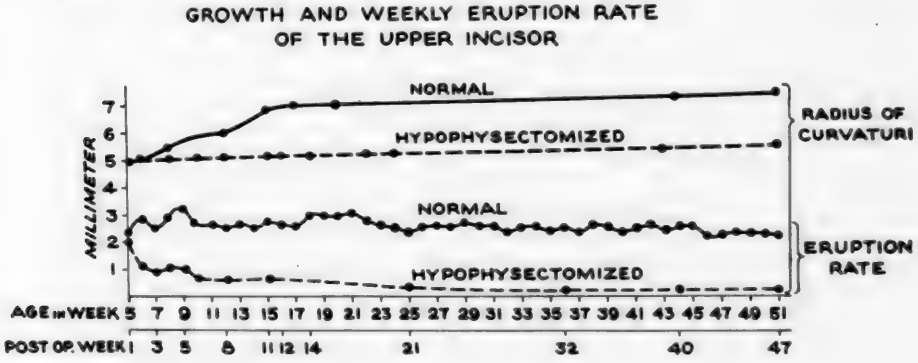

Fig. 1.

The rate of eruption recorded during fifty-one consecutive weeks shows a periodic variation and a decreasing gradient with increasing age. The maximum average of 2.73 mm. is reached at 63 days of age when, after sexual maturation, a very slow decrease is setting in. The weekly rate of eruption during 329 days (47 weeks) averages 2.51 mm. Since the dimensions of the incisors are getting progressively larger, it is to be inferred that not the entire bulk of newly formed tooth structure is extruded.

2. By comparing the incisal measurements of the hypophysectomized rats of Group II with those of the normal control Group I (Table I), it can be seen that when the rats are at the age of 45 days the values of the operated group begin to lag behind the normal averages. This indicates a stunting of dental growth sixteen days after operation. Table II and Fig. 1 show that this is accompanied by continued drop in the weekly eruption rate from 2.0 mm. to 1.1 mm. At this time, however, dentinogenesis seemingly is proceeding normally as no demarkation line or any change in the calcification picture of the newly formed dentin occurs.

Fig. 2.

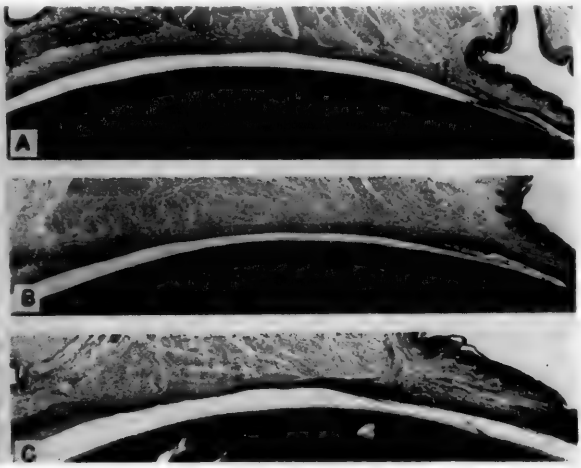

Fig. 3.

Fig. 2.—The enamel organ of the upper incisor of a hypophysectomized rat, fourteen days after operation, shows evidence of stunted growth and depressed histodifferentiation at the basal side of the loop. Note extension of uncalcified matrix of mantle dentin into the loop and osteogenic aplasia at the alveolar fundus. Original magnification, ×121; reduced ½. Specimen 9644, Plate 2044.

Fig. 3.—The labial and incisal portions of a normal and two hypophysectomized rats are shown. Original magnification, ×42; reduced ½. *A*, In the 70-day normal rat the ameloblasts extend into the gingival epithelial attachment. Note evidence of considerable osteogenic activity at the alveolar crest. Specimen 9538, Plate 2042. *B*, Forty-three days after hypophysectomy, the reduction of the ameloblasts starts beyond the alveolar crest which is aplastic. Specimen 9645, Plate 1998. *C*, Seventy-seven days after hypophysectomy, a thin layer of reduced enamel epithelium extends to the infected gingival pocket. Specimen 9663, Plate 2041.

The earliest histologic abnormalities may be observed at the apical region after a postoperative interval of fourteen days (Fig. 2). The apical zone free of dentin is definitely shorter than normal, and the odontoblastic layer shows decreased size. Furthermore, the proliferation enamel epithelium at the basal side of the cervical loop presents a cluster of cells of equal size due to delayed histodifferentiation. The enamel organ at the sites more advanced in development appears to be normal. The periosteum of the alveolar fundus, however, lacks the regular signs of rapid osteoclasia, indicating stunted dental growth.

Between the thirty-fifth and forty-second postoperative days, while almost no increase in size of incisors is recorded (Table I), another marked drop in the weekly eruption rate to 0.63 mm. has occurred (Fig. 1). According to Table III, 2B, this amounts to a 76 per cent decrease at the end of the tenth postoperative week.

In the histologic sections of rats sacrificed forty-three, fifty-six, and seventy-seven days after operation, a progressive shortening of the life span of the enamel epithelium can be recognized. Whereas, in a normal 70-day-old rat (Fig. 3, *A*) the ameloblastic layer extends almost to the free gingival margin, in the hypophysectomized animal, forty-three days postoperatively, it has atrophied down to the alveolar crest (Fig. 3, *B*); seventy-seven days after operation it is present only in the apical third. The incisal half is covered by a progressively narrower layer of reduced enamel epithelium with which the oral epithelium is fused (Fig. 3, *C*). The alveolar crests of the operated rats remain delicate due to osteogenic aplasia.

Seventy-seven days after hypophysectomy, the pathologic changes in the apical portion of the enamel organ have further progressed (Fig. 4). The cervical loop, which shows reduced histogenic activity, is bent almost rectangularly to the tooth axis. A massive layer of uncalcified predentin extends perpendicularly into the angle formed by apical epithelial diaphragm. At some distance, enamel matrix is deposited, and calcification of the adjacent predentin has set in. At the lingual enamel-free side, the situation of dentinogenesis is similar. The Hertwig's epithelial sheath is short, and oriented at a right angle to the sturdy splint of predentin. The periapical connective tissues show signs of encroachment. A striking decrease in vascularity is noticed. Due to deficient osteogenic activity, the bony fundus has become extremely thin. Below the cervical loop a perforation has occurred so that mucosal tissue of the nasal cavity penetrates into the dental alveolus. The pulpal lumen of the incisor is decreased concomitant with a thickening of the dentinal walls.

Between the 104th and 147th days following the operations, the eruption rate reached the minimum level of 0.24 mm. (Table II). In the sections of rats, 133 days after hypophysectomy, an aggravation of the histogenetic defects is noticed (Fig. 5). The apical dentin has a wavy contour and shows some irregularities in the calcification pattern as the fusion of the individual calcospherites remains incomplete for a great depth. Dentinogenesis at the central pulpal portions, however, proceeds more regularly and results in a thickening of the walls. The presence of ameloblasts is restricted to the apical fifth, while the rest of the enamel surface is covered by a decreasingly narrower layer of reduced enamel epithelium extending with a singular, very fine layer to the epithelial attachment. The pulp shows marked decrease in vascular and cellular content.

The typical folding of the apical dentin and enamel of a rat 164 days after hypophysectomy is demonstrated in Fig. 6, *A*. At a higher magnification (Fig. 6, *B*), no enamel matrix is discernible in the depth of the fold where the enamel epithelium has atrophied. Dentinogenesis at this level is equally impaired as an odontoblastic layer (*a*) is absent. Calcification of the apical dentin is very irregular. The extreme end of the labial dentin is deflected inward while degenerative changes can be observed in the ameloblastic layer adjacent to this bend (arrow). The cervical loop shows little evidence of proliferative activity, and lacks a differential arrangement of the cells.

Fig. 4.—The apical third of an upper incisor of a hypophysectomized rat, seventy-seven days after operation, shows deflected cervical loop and thickening of the labial dentinal wall. The alveolar fundus is perforated. Original magnification × 37; reduced 2/5. Specimen 9663, Plate 1999.

Fig. 5.—One hundred thirty-three days after hypophysectomy, stunting of odontogenesis and decrease in pulpal and periodontal vascularization are evident. *X*, Artificial space due to preparation. Original magnification, ×88; reduced 2/5. Specimen 9640, Plate 2043.

Fig. 6.—*A*, One hundred sixty-seven days after hypophysectomy, apical folding has occurred. Original magnification, ×37; reduced 2/5. Specimen 9647, Plate 2005. *B*, At a higher magnification the sequela of the folding phenomenon is seen. The arrow points to a circumscribed area of ameloblastic atrophy. At *a* no odontoblasts are present. *X*, Artificial space. Original magnification, ×105; reduced 2/5. Specimen 9647, Plate 1996.

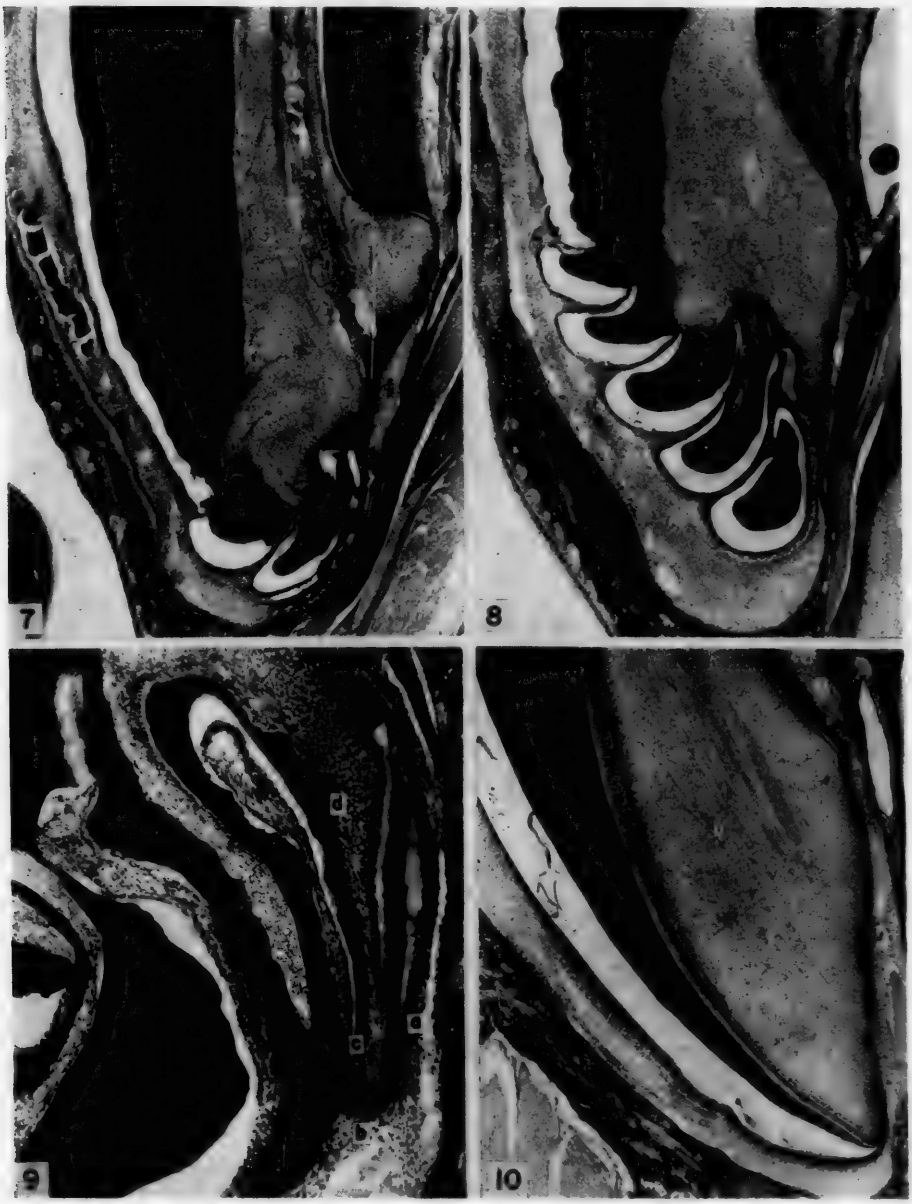

Fig. 7.—Two hundred eighty-one days after hypophysectomy, progressed apical folding and thickening of the dentinal walls are perceptible. Original magnification, ×37; reduced ⅔. Specimen 9648, Plate 2001.

Fig. 8.—Three hundred eighty-seven days after hypophysectomy, complete displacement of the cervical loop (*x*) and crumpling of the apical third has occurred. Original magnification, ×37; reduced ⅔. Specimen 8766, Plate 2003.

Fig. 9.—The atrophy of the odontogenic tissues in an incisor 400 days after hypophysectomy is shown: (*a*) dystrophy of ameloblasts, (*b*) absence of enamel formation, (*c*) arrest of dentinogenesis, (*d*) odontoblastic aplasia. Original magnification, ×110; reduced ⅔. Specimen 8759, Plate 2012.

Fig. 10.—An incisor of a rat, 347 days after hypophysectomy, reveals the pattern of odontogenesis in the absence of folding. Original magnification, ×37; reduced ⅔. Specimen 8767, Plate 2007.

3. The comparison of the measurements in Table I of the increasingly older hypophysectomized animals (Group III) with their approximate normal age controls demonstrates dwarfness of the incisal dimension in the operated group. Their radius of curvature is only 0.35 mm. greater than before operation, in contrast to the 2.4 mm. gain of the normal controls. The discrepancy in the weekly eruption rate is equally extreme (0.18 mm. in contrast to 2.3 mm. normal).

Sections of the incisors 281 days after hypophysectomy (Fig. 7) and 387 days following the operation (Fig. 8) reveal progressive apical folding according to a uniform pattern: Early atrophy of a small circumscribed area of inner enamel epithelium (Fig. 9, *a*) is accompanied by agenesis or dystrophy of enamel formation (*b*). Arrest of dentinogenesis at the site level of enamel aplasia and severely impaired calcification of this primarily deposited dentin (*c*) is associated with a localized atrophy of the adjacent odontoblasts (*d*). Once this disorganization has started, it progress further. In some specimens the apical foramen was displaced by 180 degrees into the lingual periodontal space. The severe disturbance of dentinogenesis, however, is restricted to the mantle dentin in the apical region, while at the pulpal surfaces continuous dentin formation finally obliterates the pulpal space. This proximal dentin shows irregular stratification and increase in density.

The apical distortions are contrasted in 40 per cent of the animals of Group III by more regular tooth formations, as exemplified in Fig. 10. The deviations are restricted to a little-differentiated and inward displaced cervical loop and an early atrophy of the enamel epithelium at the apical fifth. The pulp chamber of the incisal half is obliterated as the result of continuous dentin apposition, particularly at the labial side. The resting pulpal and periodontal structures show reduced vascularity; the cementum is increased in width.

The computation of individual tooth and eruption measurements of the entire group of old hypophysectomized rats throws interesting light on the relationship between dental growth and eruption. It can be seen in Table IV that there exists a strict correlation between the radius of curvature, the rate of eruption, and the incidence of apical folding. Eruption ceases almost completely in the presence of severe apical distortion, and the radius of curvature compares with that of a 45-day-old normal control. In the absence of folding, eruption proceeds at an average rate of 0.55 mm. weekly, and the radius of curvature equals that of a sixty-day normal incisor.

DISCUSSION

Hypophysectomy performed in 28-day-old rats effected a rapid and progressive reduction of the eruption rate and a drastic stunting of the growth of the upper incisors leading to an almost complete arrest of both processes after long postoperative intervals. While there occurred a gradual disturbance of amelogenesis, dentinogenesis and cementogenesis were less affected.

These findings compared with those in thyroidectomized animals lead to some conclusions with regard to the influence of the hypophysis on eruption as well as the relationship between dental growth and eruption.

1. *Influence of the Hypophysis on Eruption.*—The removal of the pituitary gland brings the entire internal secretory system in discordance due to a progressive atrophy of all endocrine glands except, possibly, the parathyroids.[13, 15]

The panglandular syndrome of hypophysectomy conceivably is responsible for a comparatively more prompt and more drastic effect on dental growth and eruption than was observed after thyroidectomy.[2] Individual data, in fact, showed that in the thyroidectomized rats there occurred no progressive deceleration of eruption such as was observed in the hypophysectomized rats in the sixty-three day period after operation. During this same interval, the size of the incisors of rats thyroidectomized at birth reached an average radius of curvature which compares with a twenty to twenty-four day normal growth period, while that of the rats hypophysectomized at 28 days did not exceed the range of a twelve- to seventeen-day normal growth period even after very long postoperative intervals.

The histologic changes in the enamel organ of the incisors of hypophysectomized rats are comparable to those of thyroidectomized ones of an equal postoperative interval. This is particularly true with reference to the shortening of the life span of the ameloblasts; nevertheless, delay in histodifferentiation and growth distortion of the cervical loop were less conspicuous. The fact that these symptoms appeared in the older hypophysectomized animals may be reconciled with a gradual and not instantaneous atrophy of the thyroid after hypophysectomy.[13]

Similar situations seem to prevail with the other pituitary target organs.[15] Concurring with the observations of Held, no appreciable change in the calcification picture during the early postoperative period was evident. In old hypophysectomized animals, however, irregularities occurred and were obviously related to the folding of the dental tissues of the apical third. The relatively long interval of 150 postoperative days until the first folding was manifested seems to indicate that possibly the hormone of a secondarily atrophied endocrine gland, rather than of the pituitary, is responsible. Ablation of the thyroid has not been seen to engender apical buckling, although the observations never could be extended over 140 days because of the imbecility of the animals and the related difficulties in keeping them alive. Neither could any nutritional depletion experiment produce similar dental changes, with the exception of Mg deficiencies.[6] Recent experiments[9] show that eruption of the rat incisor ceases four to six days after onset of Mg deficiency, and folding of the apical third sets in consistently after twenty-five to thirty-five days. The histologic appearance has many features in common with that seen in hypophysectomy after long postoperative intervals. A relation of these changes to the salt and mineral metabolism is obvious, and leads to the hypothesis that an adrenal deficiency of mineralocorticoids might constitute the mutual contributing factor to the folding pathology. This inference naturally deserves further investigation.

The results of hypophysectomy demonstrate that amelogenesis and activity of the odontogenic epithelium in particular depend directly on internal secretion, whereas dentinogenesis and cementogenesis are able to proceed at a depressed rate without the pituitary hormones. The identification of the individual hormones stimulating these processes remains to be assessed with the help of replacement therapy (see the third article of this series).

2. *The Relationship Between Dental Growth and Eruption.*—According to the introductory statement, hypophysectomy is believed to produce an experimental dissociation of the growth processes from the eruption phenomena. This conclusion was arrived at by interpreting the folding phenomenon to be the result of continued growth activity of the apical dentin in the absence of any eruptive factor.[11] Taking into account the inconsistency of folding, as well as the variability in the eruption rate of old hypophysectomized animals, a somewhat different picture is obtained.

The weekly eruption rate, after a postoperative interval of 300 to 400 days, averaged 0.18 mm. Individual analysis, however, reveals a great range of variation between 0.7 mm. and 0.08 mm. depending entirely on the absence or presence of apical folding (Table IV). The study of this folding phenomenon shows that enamel aplasia and formation of hypoplastic mantle dentin create a mechanically weak spot which is likely to bend under masticatory stresses. It is of interest to note that these observations confirm a statement made by Irving,[10] studying the incisors of rachitic rats. "In the absence of enamel matrix formation . . . , calcification of the corresponding dentin does not occur." Since in incisors of hypophysectomized rats these enamel and dentin aplasias are very extensive, folding and displacement of the odontogenic epithelium takes place. In the presence of this disorganized apical growth, the eruptive movement ceases. In the absence of folding, eruption, however, persists at a slow rate. Thyroidectomy likewise was shown previously never to check eruption completely. In both instances, the minute extrusive movement originated from a continued growth potential of the tooth which did not depend on the thyroid or the pituitary glands. Eruption, therefore, cannot become effective without a basic process of accruement of new dental tissue; that is, growth. The principal difference between a permanently growing tooth and one with limited root formation lies only in the quality of the apposite tissues. In the case of a rat incisor, eruption requires a continuous production of all tooth substances including enamel and pulpal tissues; in the instance of teeth with closing apices, eruption involves a growth spurt of radical dentin and alveolar bone.[3]

SUMMARY

The growth and eruption of the upper incisors of intact and hypophysectomized female rats of the Long-Evans strain has been studied metrically and histologically.

1. In intact rats a rapid increase in the radius of curvature was terminated at 120 days of age to be followed by a slow but steady gain until old

age. The weekly eruption rate averaged 2.53 mm., and showed periodic variation and a decreasing gradient with increasing age.

2. Hypophysectomy effected an immediate drop of the eruption rate to one-half of the normal average; in ten weeks it was progressively reduced 76 per cent.

Stunting of dental growth was noticed two weeks after operation so that the size of the incisor of 500-day-old operated animals never exceeded that of 40- to 50-day-old normal controls.

Apical folding occurred in 60 per cent of the operated animals 160 days after operation. It showed a uniform histopathologic sequence: (1) dysgenesis of inner enamel epithelium with enamel aplasia, (2) disturbed calcification of mantle dentin, (3) agenesis of odontoblasts. Possibly under the influence of masticatory stresses, the little-consolidated apical structures became folded and gave rise to displacement of the odontogenic epithelium. Its pathogenesis seems to be involved in the panglandular syndrome of hypophysectomy.

3. In the old hypophysectomized rats, the unfolded incisors were comparatively larger and maintained a minimal eruption rate (0.55 mm.) in contrast to the folded ones which were smaller and had practically ceased erupting. This shows that eruption involves a basic process of growth.

The generally more drastic effects of hypophysectomy, in comparison to thyroidectomy, point to the action of a specific pituitary factor in addition to thyrotropin, which is to be identified with substitution therapy.

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HORMONAL CONTROL OF TOOTH ERUPTION

III. The Response of the Incisors of Hypophysectomized Rats to Growth Hormone, Thyroxin, or the Combination of Both

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THE objective of this third experiment is to identify with the help of replacement therapy the specificity of the hormonal factors controlling tooth eruption. On the basis of the evidence gained in the previous experiments[2, 3] it was thought worth while to assess the response of the incisors of hypophysectomized rats to growth hormone and thyroxin and the combination of both.

The effects of substitution therapy on somatic and skeletal development have been reported recently.* However, to our knowledge only a very few studies are concerned with its effects on dental development. Following Downs'[6] pioneering experiment, Schour and Van Dyke,[8] in 1932, treated hypophysectomized rats with a polyvalent hypophyseal extract to obtain a 0.4 mm. increased weekly eruption rate. Collins, Becks, Asling, Simpson, and Evans,[5] using for the first time a pure growth hormone, found that its chronic administration to hypophysectomized rats maintained the eruption of the incisors at a rate approximately one-half of normal average. The ameloblasts showed evidence of atrophy while dentin formation proceeded at a daily rate of 10 μ in contrast to 16 μ in the normal controls.

EXPERIMENTAL ARRANGEMENT

The experimental material consists of three main groups of female rats of the Long-Evans strain.

Group I. Twenty young hypophysectomized rats, operated at 28 days of age, received injections beginning 12 days after operation for 437 days: 11 of them were injected with a daily dose of 0.5 mg. growth hormone (Group IA) and 9 of them received a daily dose of 5 γ of thyroxin (Squibb) subcutaneously (Group IB).

Group II. Twenty-seven old hyophysectomized rats, operated at 28 days of age, received injections for seven weeks, after a postoperative interval of 257 to 475 days: 10 of them were injected with a daily dose of 0.2 mg. growth hormone intraperitoneally (Group IIA), 8 of them received a daily

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*A complete review may be found under Reference 1.

injection of 7.5 γ of thyroxin, which later was reduced to 5 γ because of its toxicity (Group IIB), and 9 rats were given the combination of both hormones (Group IIC).

Group III consists of 16 intact adult rats of 192 to 222 days of age: 8 of them served as untreated controls and were sacrificed at the age of 628 to 658 days. Eight rats were injected for 436 days with an average daily dose of 1.7 mg. growth hormone. The initial dosage of 0.4 mg. was increased proportionally to the gain in body weight (Group IIIA).

Further information on the experimental arrangement concerning the periods of the study of the eruption rate and the ages at autopsy is seen in Table I. At autopsy, roentgenograms of the half skulls were made and the dimensions of the upper incisors measured with regard to their radius of curvature and thickness at the alveolar crests. Histologic sections of the upper incisors were prepared according to the method described earlier.

RESULTS

Growth Hormone Therapy.—

1. In young hypophysectomized rats the administration of growth hormone (Group IA) beginning twelve days after operation did not alter the eruption rate; after three and five weeks of therapy, the weekly averages remained the same as in the hypophysectomized controls (Table II). Following 437 daily injections of growth hormone, the actual dimensions of the incisors, however, reached approximately normal average (Table III). Each of these animals developed a malocclusion in the form of a severe maxillary prognathism; the upper incisors became more curbed than normal and their incisal ends show an irregular bevel of attrition.

Histologic analysis of the incisors shows that the enamel organ at the apical end has partially atrophied. At a higher magnification the dentin is seen directly in contact with the connective tissue of the periodontal membrane (Fig. 1, *B*). Apical folding has not occurred, although the labial dentin displays a wavy surface. The entire labial dentin is of considerable massiveness so that there remains only a very narrow pulpal canal, as is seen in all sagittal central sections. Striation and hypercalcification are characteristic of the dentin. The enamel epithelium has also atrophied in the incisal half. The alveolar bone shows evidence of great osteogenic activity which has resulted in a marked sclerosis. It is noticed that the entire apical portion of the incisor has lost its regular relationship to the nasal cavity and is displaced into the lateral bulk of the maxillary bone.

2. In old hypophysectomized rats (Group IIA), growth hormone injections did not evoke any appreciable acceleration in the rate of eruption nor did it prevent apical folding. During the seven weeks of treatment, the average totaled 2.2 mm. (0.31 mm. weekly average) in comparison to 2.1 mm. in the hypophysectomized controls (Table IV). Concurring with previous observations,[3] extreme apical folding was found in 60 per cent. The seven weeks' eruption rates of the folded incisors vary between 0.5 mm. and 1.8

TABLE I

EXPERIMENTAL ARRANGEMENT OF EXPERIMENT III

GROUP	NUMBER OF RATS	AGE AT OPERATION (DAYS)	DAILY DOSE EXCEPT SUNDAYS	PERIOD OF TREATMENT		PERIOD OF ERUPTION STUDY		AGE AT AUTOPSY (DAYS)
				DAYS	WEEKS	DAYS	WEEKS	
IA, Young hypophysectomized, growth hormone	11	28	0.5 mg. growth hormone	40-477	62.4	49-84	5	478
IB, Young hypophysectomized, thyroxin	9	28	5 γ thyroxin	40-478	62.3	49-112	9	---
IIA, Old hypophysectomized, growth hormone	10	28	0.2 mg. growth hormone	257/406-306/455	7	257/406-306/455	7	306/455
IIB, Old hypophysectomized, thyroxin	8	28	7.5 γ and 5 γ thyroxin	257/475-306/524	7	257/475-306/524	7	306/524
IIC, Old hypophysectomized, combination	9	28	0.2 mg. growth hormone, 5 γ thyroxin	259/405-308/454	7	259/405-308/454	7	308/454
IIIA, Normal, growth hormone	8	--	Average 1.7 mg. growth hormone	192/222-628/658	62.3	425/454-537/566	16	628/658
III, Normal control	8	--	----	----	---	424/739-536/851	16	629/859

TABLE II

ERUPTION RATE IN YOUNG HYPOPHYSECTOMIZED RATS FOLLOWING HORMONE THERAPY

GROUP	NUMBER OF RATS	THERAPY ONSET (DAYS)	3 WEEKS (39TH-60TH DAY)			5 WEEKS (39TH-79TH DAY)			10 WEEKS (39TH-109TH DAY)		
			AVERAGE MM.	STANDARD DEVIATION	PER CENT	AVERAGE MM.	STANDARD DEVIATION	PER CENT	AVERAGE MM.	STANDARD DEVIATION	PER CENT
IA, Hypophysectomized + growth hormone	9	40	1.28	±0.134	56	1.21	±0.161	45	---	----	---
IB, Hypophysectomized + thyroxin	8	40	1.62	±0.146	70	1.58	±0.141	60	1.58	±0.145	60
*Hypophysectomized	9	--	1.3	±0.177	56	1.21	±0.155	45	0.63	±0.037	24
*Normal	10	--	2.3	±0.09	100	2.73	±0.100	100	2.66	±0.134	100

*According to Table III, second article of this series.[3]

TABLE III

INCISOR DIMENSION AT AUTOPSY AND AVERAGE ERUPTION RATES DURING SIXTEEN WEEKS IN GROWTH-HORMONE-TREATED RATS

GROUP	NUMBER OF RATS	AGE AT AUTOPSY (DAYS)	PERIOD OF TREATMENT (DAYS)	THICKNESS OF INCISOR		RADIUS OF CURVATURE		WEEKLY ERUPTION RATE	
				AVERAGE MM.	STANDARD DEVIATION	AVERAGE MM.	STANDARD DEVIATION	AVERAGE MM.	STANDARD DEVIATION
IA, Hypophysectomized + growth hormone	5	477	437	2.94	±0.173	7.2	±0.173	1.2	±0.16
IIIA, Normal + growth hormone	8	647	436	3.35	±0.224	8.3	±0.151	2.2	±0.40
III, Normal control	5	640	---	3.16	0.100	7.4	±0.180	2.3	±0.27

mm., while those of the unfolded ones range between 4.1 mm. and 4.7 mm. The dimensions behave accordingly, being larger in the unfolded incisors. As a group average they exceed the untreated control group, indicating the occurrence of some growth.

TABLE IV

ERUPTION RATE AND INCISOR DIMENSIONS IN OLD HYPOPHYSECTOMIZED RATS FOLLOWING HORMONE THERAPY DURING SEVEN WEEKS

GROUP II		NUMBER OF RATS	TOTAL ERUPTION RATE AFTER 7 WEEKS (MM.)	INCISOR THICKNESS (MM.)	RADIUS OF CURVATURE (MM.)
IIA, Hypophysectomized + growth hormone	Group average	9	2.2 ±1.59	2.26 ±0.193	5.66 ±0.416
	Not folded	3	4.43 ±0.292		5.9 ±0.100
	Folded apex	6	1.13 ±0.43		5.23 ±0.275
IIB, Hypophysectomized + thyroxin	Group average	8	4.4 ±2.50	2.17 ±0.107	5.61 ±0.253
	Not folded	3	7.16 1.17		5.9 ±0.100
	Folded apex	5	2.72 1.03		5.44 ±0.132
IIC, Hypophysectomized + combination	Group average	9	5.7 ±3.17	2.24 ±0.112	5.8 ±0.310
	Not folded	3	9.5 ±0.173		6.1 ±0.158
	Folded apex	6	3.73 ±1.56		5.6 0.215
*Hypophysectomized control	Group average	10	2.1 ±1.60	2.13 ±0.01	5.39 ±0.213
	Not folded	4	3.9 ±0.62		5.57 ±0.100
	Folded apex	6	0.9 ±0.289		5.26 ±0.489

*According to Table IV, second article of this series.[3]

Histologically, in the folded incisors a great tendency toward repair is noticed. In Fig. 2 at *x*, a relocation of the odontogenic tissues into proper position is seen. Amelogenesis as well as dentinogenesis consequently shows an improved pattern. The surrounding alveolar bone gives evidence of resumed osteogenic activity (*Y*). In the absence of folding (Fig. 3), this growth of the alveolar fundus is equally conspicuous. The accrued apical dentin does not undergo thickening. The pulp tissue in the region of the odontogenic epithelium displays evidence of marked cellular increase.

3. Chronic administration of growth hormone to intact adult rats (Group IIIA) again has no stimulating effect on eruption; the weekly rate is even somewhat lower than normal (Table III). This is in contrast to the striking increase in tooth size of these "giant" rats.

Histologically, increased mitotic activity of the apical pulpal parenchyma is discernible (Fig. 4). The pulp is highly vascularized. Dentinogenesis exhibits a normal pattern producing regular-sized dentinal walls. Ameloblastic activity is stimulated as the complete unreduced enamel organ extends near the free gingival margin (Fig. 5, *A*). Osteogenesis is activated notably at the alveolar crest.

Thyroxin Therapy.—

1. Thyroxin administered chronically to young hypophysectomized rats (Group IB) accelerated the weekly eruption rate 36 per cent in a ten-week period; it averaged consistently 1.6 mm. per week, and did not decrease to

0.6 mm., as did the hypophysectomized rats, the untreated controls, and the growth-hormone-treated rats (Table II).

2. On receiving daily injections of thyroxin, old hypophysectomized rats (Group IIB) also resumed eruption at an accelerated rate; in seven weeks it averaged 4.4 mm. (Table IV) in contrast to 2.1 mm. for the untreated group. The standard deviation is high (±2.5) because of the small response of the incisors with folding. The latter showed a seven weeks' total of 2.72 mm. in contrast to 7.16 mm. of the unfolded ones.

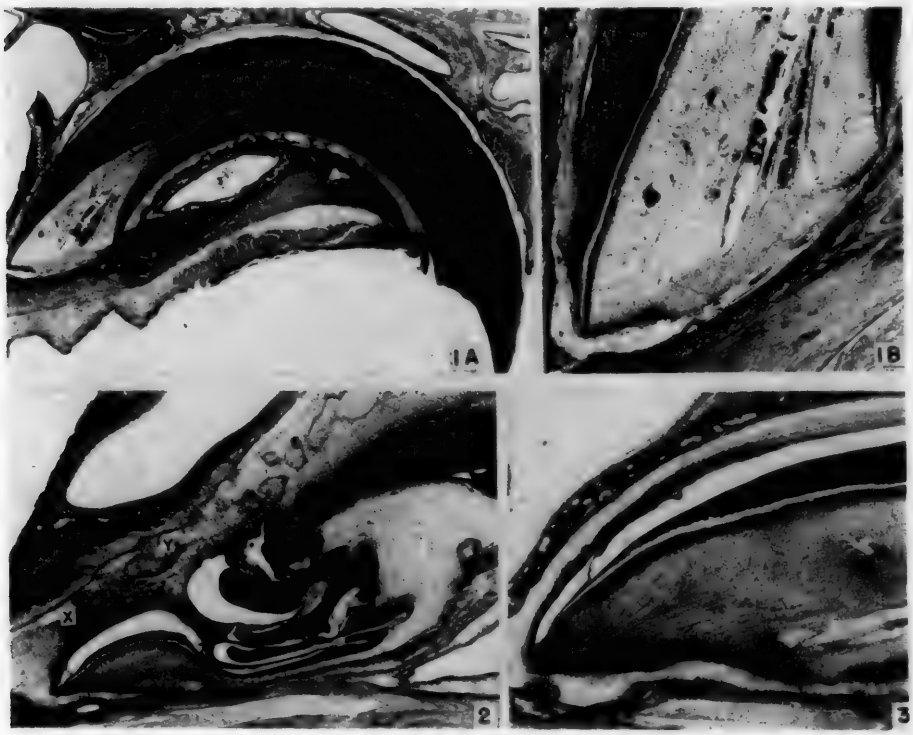

Fig. 1.—*A*, The central section of the incisor of a hypophysectomized rat, which was injected for 437 days with growth hormone beginning 12 days after the operation, shows no enamel organ present at the apical fifth. Original magnification, ×11; reduced 6/11. Specimen 9022 I, Plate 2052.

B, The apical region is shown at a higher magnification. Note disturbed dentin calcification. Original magnification, ×42; reduced 6/11. Specimen 9022 II, Plate 2029.

Fig. 2.—Basal portion of the incisor of a rat, hypophysectomized at 28 days of age, which was given growth hormone for 7 weeks beginning 428 days after operation, shows the effect of growth hormone substitution. The apex has grown behind the folded portion (*x*). *Y*, increased osteogenic activity. Original magnification, ×42; reduced 6/11. Specimen 8775, Plate 2015.

Fig. 3.—Unfolded incisor of a hypophysectomized rat injected for 7 weeks with growth hormone, beginning 280 days after operation, shows marked proliferation of the apical pulpal parenchyma. Original magnification, ×42; reduced 6/11. Specimen 8771, Plate 2017.

The incisor dimensions, notably the radius of curvature, compare favorably with those of the growth-hormone-treated group, except that the folded incisors show some greater response.

The histologic section shown in Fig. 6 demonstrates that thyroxin is not able to reverse effectively severe pituiprivic derangement of the odontogenic tissues, although some proliferation of odontogenic epithelium and formation of new mantle has occurred. More favorable changes have taken place in the slightly folded incisor shown in Fig. 7, as the newly formed structures appear to be of a regular pattern. There is a remarkable increase in vascularity of the pulpal and periodontal tissues, while the alveolar plates are very thin. The alveolar crests display little osteogenic activity; the adjacent enamel organ consists of an extremely thin layer of reduced enamel epithelium (Fig. 5, *B*).

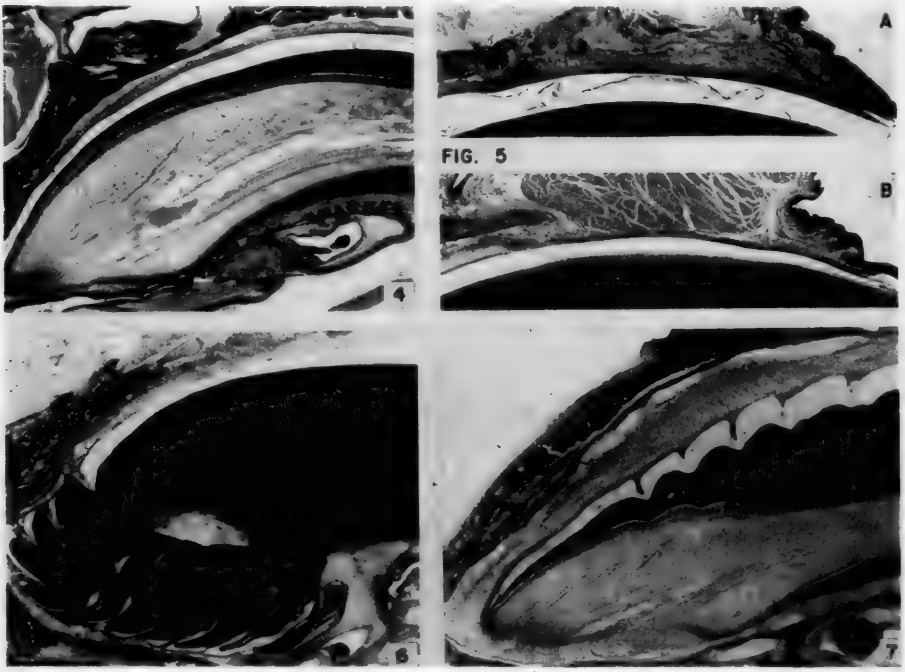

Fig. 4.—Following 437 days of injections of growth hormone to an intact rat, 658 days of age, the incisor shows increased proliferation of the apical parenchyma of the pulp and normal tooth structures. Original magnification, ×18; reduced 4/7. Specimen 8984, Plate 2060.

Fig. 5.—The labial incisal portion of the periodontal tissues of two different experimental rats is shown. Original magnification, ×36; reduced 4/7. *A*, Following chronic injection of growth hormone to an intact rat, a complete enamel organ extends to the free gingival margin. Note marked osteogenesis at the alveolar crest. Specimen 8355, Plate 2053. *B*, The thyroxin-treated old hypophysectomized rat shows no regeneration of the enamel epithelium subjacent to the aplastic alveolar crest. Specimen 8784, Plate 2055.

Fig. 6.—Severely folded incisor of an old hypophysectomized rat which was treated with thyroxin for 7 weeks, beginning 497 days after operation, shows little response. Original magnification, ×42; reduced 4/7. Specimen 8782, Plate 2017.

Fig. 7.—Little-folded incisor of a hypophysectomized rat which was administered thyroxin for 7 weeks, beginning 280 days after operation, shows restoration of the odontogenic processes. Original magnification, ×42; reduced 4/7. Specimen 8779, Plate 2018.

Combined Growth Hormone and Thyroxin Therapy.—

The greatest acceleration of tooth eruption in old hypophysectomized rats is obtained by combined growth hormone and thyroxin therapy (Group

IIC, Table IV). The average eruption rate during the seven weeks' treatment period totaled 5.7 mm. ±0.17. The high standard deviation again is due to a much greater response of the three unfolded incisors (9.5 mm.) in comparison to the six folded ones (3.7 mm.). The latter, however, range still higher than their equivalents in the other treated groups. The tooth dimensions likewise show the highest values recorded in Group II (Table IV), notably also among those of folded incisors.

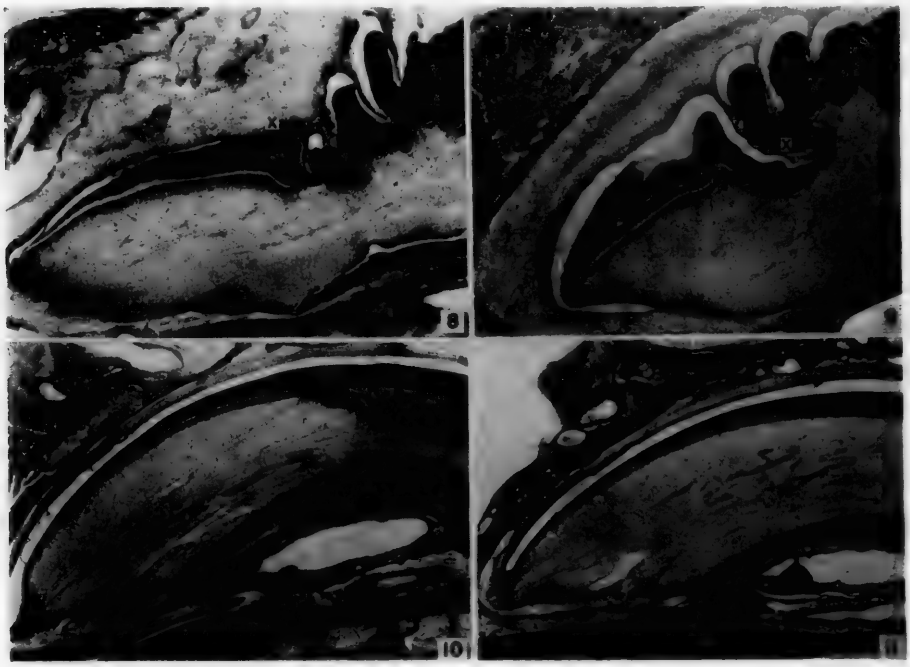

Fig. 8.—Severely folded incisor of an old hypophysectomized rat receiving the combined therapy of growth hormone and thyroxin for 7 weeks, beginning 302 days after operation, exhibits complete restoration of the enamel organ. *X*, Site of total degeneration of enamel organ. *Y*, Osteogenesis. Original magnification, ×42; reduced ⅓. Specimen 8791, Plate 2026.

Fig. 9.—Resumption of regular amelogenesis upon administration of combined therapy for 44 days to a rat 408 days after hypophysectomy is evidenced. *X*, Site of total atrophy of enamel organ. Original magnification, ×42; reduced ⅓. Specimen 6898, Plate 2056.

Fig. 10.—The unfolded incisor of an old hypophysectomized rat responds with normal odontogenesis on 7 weeks combined therapy beginning 352 days after operation. Original magnification, ×18, reduced ⅓. Specimen 8790, Plate 2058.

Fig. 11.—The basal portion of an intact 639-day-old rat shows a large well-differentiated cervical loop of the enamel organ. Original magnification, ×18; reduced ⅓. Specimen 8980, Plate 2030.

The photomicrographs shown in Figs. 8 and 9 demonstrate that combination therapy elicits amazing reparative changes in severely folded incisors. They include a gradual resumption of amelogenesis, which previously had completely subsided (Figs. 8 and 9, "*X*"). Dentinogenesis is greatly stimulated as far as matrix formation is concerned; calcification, however, is somewhat deficient. The unfolded incisor (Fig. 10) resumed an aspect almost indistinguishable from normal (Fig. 11) except for a somewhat less-differentiated epithelial loop.

DISCUSSION

An analysis of these data, together with those of the previous two experiments,[2, 3] reflects on the specificity of the hormones controlling eruption as well as on the mechanism of eruption itself.

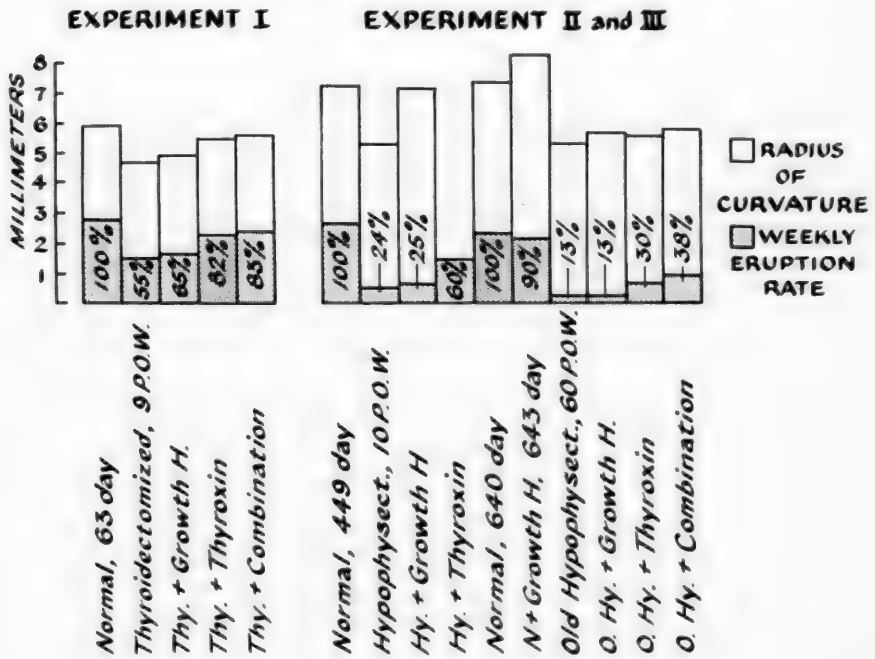

Fig. 12.

Two main principles of endocrine influence have been disclosed. From the ablation experiments we have learned that the removal of the pituitary gland has a more prompt and more drastic effect on eruption than thyroidectomy. From the replacement experiments it became evident that the pituitary gland influences eruption not only with its thyrotropin but also with a specific substance of somatic electiveness, the pituitary growth hormone. For the purpose of comparison of the various experimental effects on dental development, average measurements of tooth size (radius of curvature) and weekly eruption rate of each animal group of the three experiments have been plotted graphically in Fig. 12.

The effects of thyroid hormone on dental growth and eruption are quantitatively and qualitatively different from those of growth hormone. Quantitatively, thyroxin proves to be the essential factor, stimulating the eruptive movement and at the same time increasing the tooth size, while it has little influence on alveolar growth. Growth hormone, in contrast, spurs dental as well as alveolar growth, but leaves the eruption rate unchanged. The quali-

tative aspect was elucidated by the histologic study of the reparative changes in the thyroprivic or pituiprivic incisors evoked by specific hormone therapy. Thyroxin, the hormone of skeletal maturation, enhances, generally, cell metabolism and tissue vascularization, while it activates specifically the odontogenic epithelium to induce differential growth of the dental and peridental tissues. Growth hormone, the protein builder, which generally has a stimulative effect on growth and maintenance of the mesenchymal derivatives, cartilage in particular, also promotes membranous osteogenesis and dentinogenesis, as well as pulpal proliferation. It thereby enlarges the size of the incisor without augmenting the eruption rate because the accrued dental tissues are invested continuously into the stimulated alveolar bone. Growth hormone, in contrast to the thyroid hormone, thus is inept to induce a differential growth rate of the dental and peridental tissues. This explains why the eruption of the hypophysectomized as well as intact rats after chronic treatment with growth hormone remained unchanged although the tooth size increased markedly.

Due to the particular electiveness of both hormones, combination therapy had a synergistic effect in both the thyroidectomized and the hypophysectomized rats, but to a variable degree. In the thyroprivic state, in which some pituitary growth hormone is still produced, the depressed eruption rate responded only little more to combination therapy than to thyroxin alone. In the pituiprivic state, in which the thyroid gland is atrophied, it elicited optimal effect on the eruption rate as well as the histology. The fact that it engendered almost complete restoration of the extreme atrophic odontogenic organ complements the evidence obtained in the two previous experiments that thyroxin together with growth hormone presides over the eruption process. This synergism of growth hormone and thyroxin was also experienced in the development of the long bones,[4] and might be kept in mind when therapeutic measures are attempted, particularly in severe hypothyroid cases with generalized delayed development and eruption of the tooth germs.

Although the present studies deal primarily with the stimulative factors in dental growth and eruption, the possibility of a depressor action of some endocrine glands should not be overlooked. The decreased eruption rate of the intact animals after sexual maturation might indicate such an influence of the sex hormones. The antigrowth effects of ACTH are well established in bone development. No investigation has been made so far of accessory endocrine factors, such as the cortical steroids in the eruption picture. It is entirely possible that other endocrine glands, by virtue of their effects on metabolic interrelations, also affect tooth development and eruption in one way or another.

Eruption may thus be defined as the last phase of topogenesis* of the alveodental organ. It involves a basic process of proliferation of the dental structures, which is governed by the pituitary growth hormone. Eruption, however, becomes effective only through a differential growth of the dental

*Topogenesis, a term used in experimental embryology, designates the sum of movements of growth which result in the formation of an organ.[1]

and peridental tissues induced by the proper interaction of the epithelial sheath. The present observations provide factual evidence that epithelium plays a "steering" role in the eruption process. The fact that this epithelial activity is controlled by the thyroid hormone relates eruption to maturation.

SUMMARY

Replacement therapy in hypophysectomized rats served to identify the individual hormones controlling their odontogenic defects. It had the same qualitative effect shortly after the operation as well as after long postoperative intervals.

1. Growth hormone resulted in an increase in size without hastening the eruption rate. The latter remained in the young as well as old groups identical with the untreated hypophysectomized controls. Histologically, a "rejuvenation" of the connective tissues, without activation of amelogenesis, was observed. Administration of growth hormone to intact animals had similar effects.

2. Thyroxin treatment increased the tooth dimensions and accelerated the eruption rate 36 per cent in the young group and 46 per cent in the old group. Amelogenesis showed an improved pattern, and the vascularization of the tissues was restored.

3. The combination of both hormones showed optimal effects on eruption rate, and growth was characterized histologically by an amazing restoration of the completely atrophied enamel organ.

These observations thus lead to the conclusion that tooth eruption is presided over by the synergism of two hormones: The pituitary growth hormone, which stimulates the basic process of growth, and the thyroid hormone, which controls differentiation or maturation.

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THE EFFECT OF ESTROGEN ON THE GINGIVA AND ALVEOLAR BONE OF MOLARS IN RATS AND MICE

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INTRODUCTION AND REVIEW OF LITERATURE

THE effect of estrogens on the skeletons of birds and various species of mammals is well known. In mammals it has been shown that there is a species difference with regard to the response of bone tissue and that the mouse is more responsive to estrogens than any other laboratory mammal.[1] Administration of estrogen produces osteosclerosis by increased endosteal bone formation in the mouse,[1-3] while in the rat osteosclerosis is the result of retarded bone resorption.[1] However, after administration of estrogens the alveolar bone of the rat does not show osteosclerotic changes, while that of the mouse reacts as all other bones.[4] The effect of estrogens varies not only in species, but also at different stages of development and in different areas of the skeleton in the same species.[3] In mice the areas of rapid skeletal growth are areas which show the maximum osteogenic response to estrogens and in old animals the response is delayed.[3]

Results of experiments on the effect of estrogens on the oral mucous membrane vary. Whereas increased hornification has been reported in the estrogen-treated monkeys,[5] no changes have been found in the oral mucosa of the mouse or the rat.[4] However, Stahl, Weinmann, Schour, and Budy have shown that estrogen administration leads to an increase in the number and size of the epithelial remnants in the periodontal membrane of mice.[4] They have further shown that the estrogens have no effect on the epithelial attachment of the rat or the mouse molars.

Although there is no conclusive evidence of beneficial effect of estrogenic hormone in certain diseases involving the human oral mucosa, the use of estradiol and diethylstilbestrol has been recommended[6, 7] in the treatment of leukoplakia.

The present investigation was undertaken to study the effect of estradiol benzoate and diethylstilbestrol (a natural and a synthetic estrogen, respectively) on the alveolar bone and especially on the epithelial attachment and the periodontal membranes of rats and mice at various age levels.

METHODS AND MATERIALS

Young Rats.—Sixteen newborn rats (Wistar) were divided into two groups. Group A consisted of eight newborn rats, used as controls, which

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were sacrificed at 5, 11, 16, 21, 26, 33, 37, and 42 days of age, respectively. Group B consisted of eight newborn rats which received subcutaneous injections of a-estradiol benzoate in olive oil and which were sacrificed at 5, 11, 16, 21, 26, 33, 37, and 42 days of age. They received a total of from 0.05 to 1.30 mg. of a-estradiol benzoate.

Young Mice.—Twenty-two newborn mice of CFI strain were divided into three groups. Group A consisted of eight newborn mice which were sacrificed at 5, 10, 15, 20, 25, 30, 35, and 40 days of age, respectively, and used as controls.

Group B consisted of six mice which were given weekly injections of diethylstilbestrol in olive oil and which were sacrificed at 5, 10, 15, 20, 25, and 30 days of age, respectively. They received a total of 0.05, to 0.80 mg. of diethylstilbestrol.

Group C consisted of eight newborn mice which were given weekly injections of a-estradiol benzoate in olive oil and which were sacrificed at 5, 10, 15, 20, 25, 30, 35, and 40 days of age, respectively. They received a total dosage of 0.05 to 1.30 mg. of a-estradiol benzoate.

Old Mice.—Twenty-three 1-year-old mice of CFI strain were divided into three groups. Group A consisted of six mice which were sacrificed from one to thirteen weeks from the start of the experiment and were used as controls.

Group B consisted of nine mice in which a sterile pellet of about 2.0 mg. of crystalline a-estradiol benzoate was implanted subcutaneously in the back, and the animals were sacrificed at one, three, four, five, five and one-half, six, seven, eight and one-half, and thirteen weeks after the start of the experiment.

Group C consisted of eight mice which received weekly injections of 0.5 mg. of a-estradiol benzoate in olive oil (total dose 1.5 to 6.0 mg.) and which were sacrificed at three, four and one-half, five and one-half, six, seven and one-half, ten, eleven, and twelve weeks, respectively, after the start of the experiment.

After sacrifice, all the animals were decapitated and radiographs were taken of bisected heads. Selected skulls were then decalcified in 5 per cent nitric acid. Following decalcification, the region of the maxillary molars with their supporting bone was dissected, embedded in celloidin, cut sagitally, and stained with hematoxylin and eosin.

RADIOGRAPHIC FINDINGS

Young Rats.—In the young rats no roentgenologic differences were noticeable in the teeth or their supporting structures between the experimental and the control animals.

Young Mice.—In young mice of Groups B and C which received up to 0.30 mg. of diethylstilbestrol or a-estradiol benzoate over a period of twenty-five days, no changes could be seen. In animals in which the administration

of estrogens was continued beyond twenty-five days and which received 0.80 to 1.30 mg. of one of the estrogenic compounds, radiographic changes could be seen in the supporting structure of molars.

In the control animals, up to 30 days of age the interdental and interradicular bony septa appeared as radiopaque areas. In animals 30 days of age a small area of radiolucency appeared in the interradicular septum of the first molar. This area of radiolucency gradually became larger and more distinct. At 40 days of age another area of radiolucency appeared in the bony septum between the first and second molars. In the experimental animals these areas of radolucency could not be seen even at 40 days of age.

Old Mice.—

Group A: In the control mice which at the beginning of the experiment were 1 year old, the molars showed considerable attrition. The apices of roots showed the normal increase of cementum which was especially marked in the first upper and lower molars. The roots were surrounded by a narrow line of radiolucency. The interdental and interradicular septa appeared radiopaque without any areas of radiolucency and they surrounded at least two-thirds of the molar roots.

Group B: In animals of Group B radiographic changes of varying degree could be noticed in six of the nine animals. In all of the affected animals a-estradiol benzoate had been implanted for at least three weeks. The radiographic changes ranged from a slight widening of the periodontal membrane to a reduction in height of the interdental and interradicular bony septa. In one case the entire interdental septum between upper first and second molars had disappeared.

Group C: In all animals of Group C radiographic changes of varying degree could be seen. In four animals these consisted of a slight widening of the periodontal membrane and a reduction in the height of the interdental septum between the first and second molars. In the remaining four animals the changes were more severe and especially marked in upper teeth. The interdental septum between the first and second molars was completely or almost completely destroyed. Radiolucent areas could also be seen in the bifurcation of first molars. In severe cases the second molars were out of alignment.

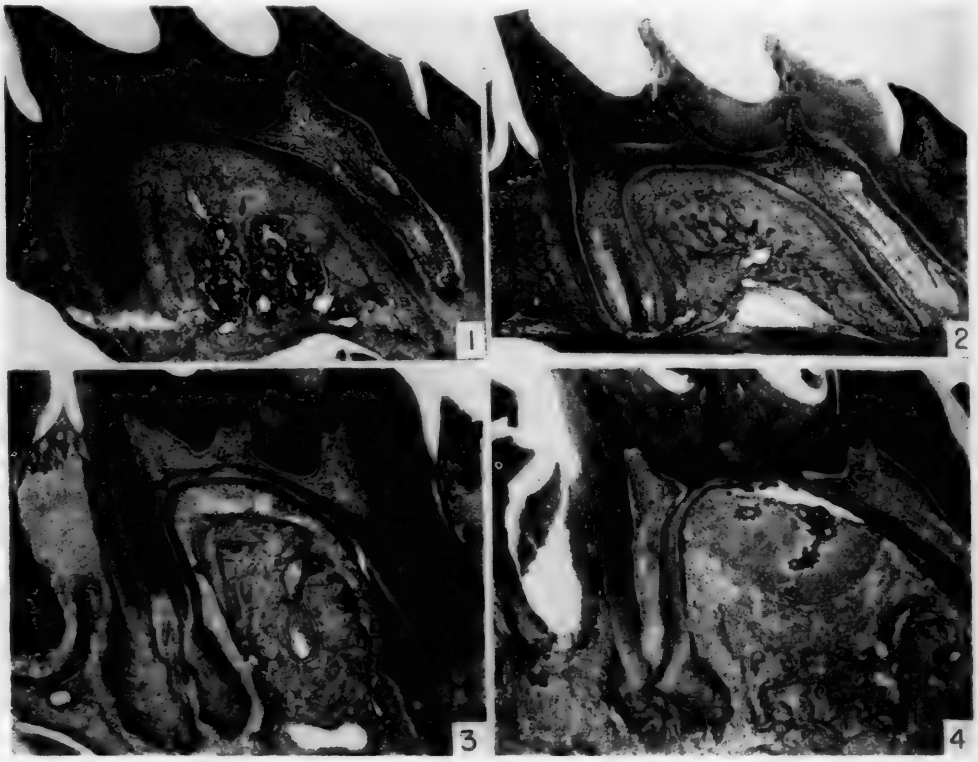
HISTOLOGIC FINDINGS

Young Rats.—Comparison of the control rats and rats injected with 0.05 to 1.30 mg. of a-estradiol benzoate showed no significant difference in the development or the structures of the molars, in the supporting structures of the teeth, in the size of the bone marrow spaces, in the type of marrow, or in the epithelial attachment.

Young Mice.—In the teeth and supporting structures of the experimental animals of Groups B and C which received 0.20 mg. of diethylstilbestrol or a-estradiol benzoate there were no significant changes. Animals which at time

of sacrifice were 25 days old or older and which received 0.80 to 1.30 mg. of diethylstilbestrol or a-estradiol benzoate showed changes in the bone marrow, supporting bone, and the periodontal membrane.

Group A: In the control animals 25 days old or older both the first and second molars had erupted. The interradicular septa of the second molar and the interdental septum between the first and second molars consisted of mature lamellated bone. The marrow spaces were few and contained fibrous marrow.



Figs. 1 and 2.—Photomicrographs of sagittal sections through the upper first molars of 30-day-old control mouse (Fig. 1) and a litter mate (Fig. 2) which received a total dose of 0.80 mg. of diethylstilbestrol. Note narrowing of marrow spaces and replacement of hemopoietic marrow in Fig. 2. (Original magnification, ×40, reduced ½.)

Figs. 3 and 4.—Photomicrographs of sagittal sections through upper first molars of 1-year-old control mouse (Fig. 3) and an experimental animal (Fig. 4) which received a total dose of 6.0 mg. of a-estradiol benzoate. (Original magnification, ×40, reduced ½.)

The interradicular septa of the first molar also consist of mature lamellated bone. In this area there were numerous wide marrow spaces which contained hemopoietic marrow (Fig. 1). The bone surrounding the marrow spaces shows either lacunae with osteoclasts or aplastic lines of inactivity.

Groups B. and C: In animals treated either with a-estradiol benzoate or with stilbestrol, the most striking changes were seen in the region of the

first molar. The interradicular septa of the first molar consisted, as in the control animals, of mature lamellated bone. However, the marrow spaces were greatly reduced in size and number. On the endosteal surface of these marrow spaces apposition of bone could be seen. The hemopoietic marrow was replaced by fibrous marrow (Fig. 2).

Old Mice.—

Group A (control): In the mice 1 year of age at the beginning of the experiment the interradicular septa of the first and second molars and the interdental septum between the two teeth consisted of matured lamellated bone (Fig. 3). The marrow spaces in the interradicular septa of first molar were very few and the marrow consisted mainly of blood vessels, on the periphery of which a few hemopoietic cells could be seen (Fig. 3). In the interradicular septum of the second molar and the interdental septum between the first and second molars, the marrow spaces were either absent or very much reduced in size.

The first, as well as the second, molar showed attrition. The apical thirds of their roots showed large amounts of cellular cementum. This was more pronounced on the first than on the second molar roots. Acellular cementum covered the gingival two-thirds of all roots (Fig. 3).

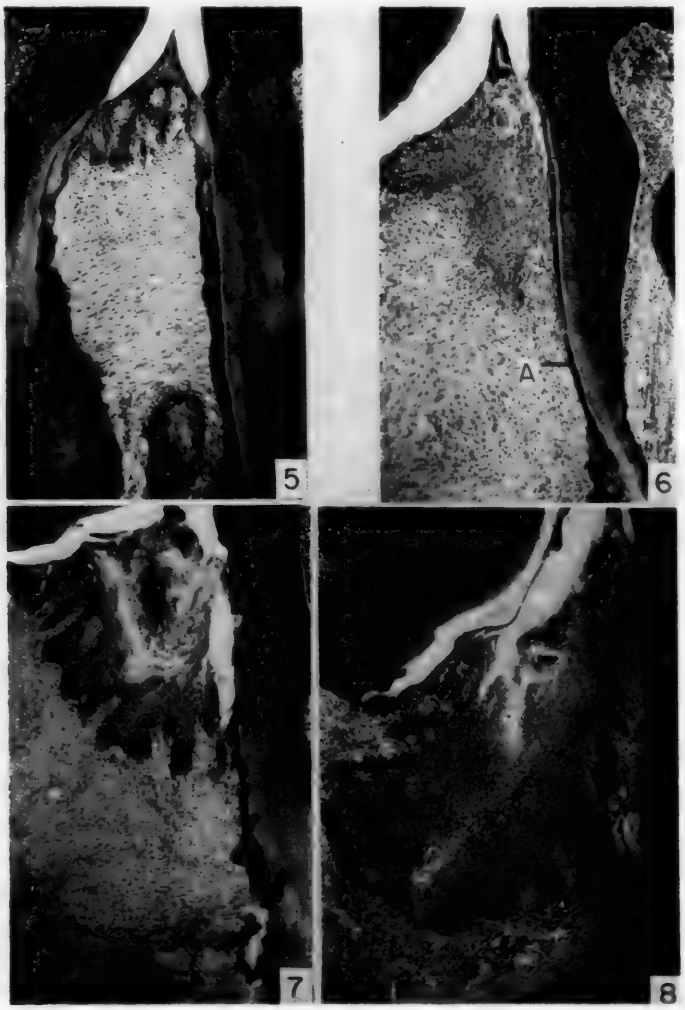
The epithelial attachment on all surfaces of the first and second molars had grown downward along the root surfaces. This apical growth had progressed farthest on the mesial surface of the first molar. However, the bottom of the gingival sulcus was on the enamel in all instances (Fig. 5). The interdental papilla and the gingiva on the mesial surface of the first molar were free of inflammatory cells.

Group B (pellet implants of estradiol benzoate): In the animal which was sacrificed three weeks after the implant, the most significant change was the downgrowth of the epithelial attachment on the root surfaces of the first and second molars. Whereas the apical end of the epithelial attachment was at about the middle of most root surfaces, on the distal surface of the first molar it had grown almost to the apical third of the root (Fig. 6). The bottoms of the crevices were on the cementoenamel junction, except on the distal surface of the first molars where it had shifted to the root surfaces. The epithelial covering of the interdental papilla between the first and second molars showed infiltration with polymorphonuclear leukocytes. In all other respects the supporting structures of the teeth were unaltered.

In the animal which was sacrificed six weeks after the implant the changes were similar, but more severe than those seen in the animal three weeks after the implant. The epithelial attachments on the distal surface of the first molar and the mesial surface of the second molar had proliferated farther apically, and the bottoms of the pockets were at the middle of the root (Fig. 7). The epithelium had also grown onto the cementum in the bifurcation of the second molar. The interdental papilla between the first and second molars was blunted and the epithelial ridges had grown deep into the underlying connective tissue (Fig. 7).

The distal surfaces of the distal roots of the first molars showed numerous areas of resorption (Fig. 7).

Group C (injections of estradiol benzoate): In old animals which had received injections of estradiol benzoate the primary changes were found in the gingiva and epithelial attachment. The effect on the alveolar bone apparently was secondary.



Figs. 5 and 6.—Photomicrographs of sagittal sections through interdental area between upper first and second molars. Fig. 5 is from control animal 1 year old and Fig. 6 from an experimental mouse which received an implant of 2.0 mg. of a-estradiol benzoate. In Fig. 6 note downgrowth of epithelial attachment to point *A* on distal root of first molar and the proliferation of gingival epithelium. (Original magnification, ×120, reduced ½.)

Figs. 7 and 8.—Photomicrographs of sagittal sections through interdental area between upper first and second molars in old mice. Fig. 7 is from an animal which received implant of 2.0 mg. of a-estradiol benzoate for six weeks. Fig. 8 is from mouse which received total of 3.0 mg. of a-estradiol benzoate over a period of five and one-half weeks. Note areas of root resorption, and a "tumor-like" proliferation of gingival epithelium. (Original magnification, ×120, reduced ½.)

Three weeks after the first injection changes could be noticed in the epithelial attachment. On the mesial surface of the second molar the epithelial attachment had grown farther along the root surface than in the control animals. Also, the attachment had grown to the surface of the cementum in the bifurcation of the second molar. This epithelium was still attached to the cementum and therefore the bifurcation was not exposed.

After six weekly injections of estradiol benzoate the changes were more marked. The epithelium of the interdental papilla between the first and second molars had proliferated into the connective tissue to a marked degree (Fig. 8). However, the epithelial attachments on the distal surface of the first molar and on the mesial surface of the second molar were not markedly altered. The interdental bony septum had undergone marked resorption. Only the apical one-fourth of the distal root of the first molar was surrounded by bone. The crest of the interdental bony septum was concave.

The epithelial attachment on the distal surface of the second molar had grown farther apically. Also, the epithelium had proliferated to a larger surface along the cementum of the bifurcation of the second molar. All the root surfaces of the molars showed areas of resorption.

After twelve weekly injections of estradiol benzoate, the epithelial attachments had proliferated to the apex of the mesial root of the second molar and almost to the apex of the distal root of the first molar (Fig. 9). Due to the separation of the epithelial attachment from the cementum, the mesial surface of the second molar root was completely exposed. Almost the entire distal root of the first molar was covered with epithelium. The periodontal membrane fibers were attached only to the apical one-fourth of the root surface (Fig. 4). The interdental papilla and the interdental septum had completely disappeared (Fig. 4).

In the bifurcation of the second molar the epithelium had grown farther along the surface of the cementum, but the most striking changes were seen in the bifurcation of the first molar roots. Here the epithelium had first proliferated onto the surface of cementum and then had separated from it, leading to the exposure of the bifurcation and the formation of a pocket (Figs. 4 and 11). The pocket was filled with polymorphonuclear leukocytes. The interradicular bony septum had undergone resorption and its crest was cupped or concave. The connective tissue between the crest of the bony septum and the interradicular pocket was infiltrated by numerous plasma cells. The plasma cell infiltration also extended into the marrow spaces of the interradicular bony septum. (Compare Figs. 10 and 11.)

Numerous areas of resorption were seen on the root surfaces of molars. These were especially marked on the distal surfaces of the distal roots of the first molars (Fig. 4).

DISCUSSION

(1) Changes in the Gingival Epithelium and Epithelial Attachment.—In young or growing mice even large doses of estrogen did not lead to any changes in the gingival epithelium or in the epithelial attachment. However, when

similar doses were given to old mice, or if estrogen pellets were implanted in old mice, the changes in the gingival epithelium and epithelial attachment were striking. In the early stages of estrogen administration the epithelial attachment proliferated along the root surfaces and also grew onto the cementum in the bifurcation of roots. This proliferation of the epithelium continued until

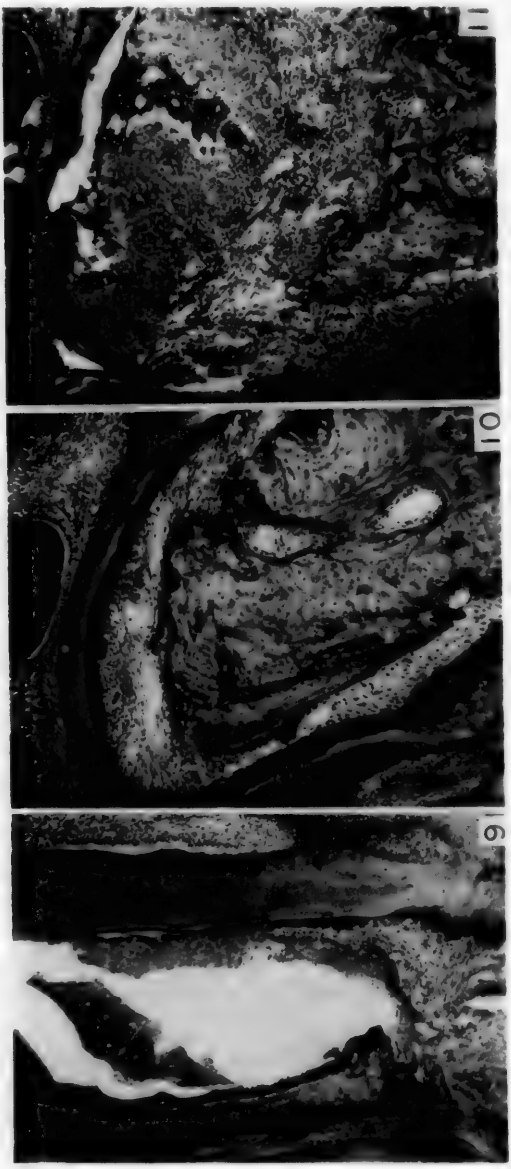


Fig. 9.—Photomicrograph of a sagittal section through interproximal area between upper first and second molars of an old mouse which received a total of 6.0 mg. of a-estradiol benzoate over a period of twelve weeks. Note exposure of second molar root and downgrowth of epithelial attachment almost to the apical part of distal root of first molar. (Original magnification, ×120, reduced ½.)

Figs. 10 and 11.—Photomicrographs of sagittal sections through the interradicular areas of first molars in old mice. Fig. 10 is from a control. Fig. 11 is from an experimental animal which received 6.0 mg. of a-estradiol benzoate over a period of twelve weeks. In the experimental mouse note epithelium in interradicular area, an interradicular pocket with numerous polymorphonuclear leukocytes, resorption and "cupping" of interradicular bony septum. (Original magnification, ×120, reduced ½.)

in animals which were treated for twelve weeks it covered almost the entire length of the distal root of the first and the mesial root of the second molars.

In addition to the downgrowth of epithelial attachment, the gingival epithelium of the interdental papilla between the first and second molars also proliferated, almost in a tumorlike fashion. Simultaneous with gingival epithelium, the conical shape of the interdental papilla was altered and it progressively became blunted and finally concave on it upper surface.

In the final stages of the experiment, the proliferated epithelium became detached from the root surfaces and from the cementum in the root bifurcations and this led to the formation of interdental and interradicular periodontal pockets. Due to the formation of these pockets, the interradicular and interdental bony septa showed resorption and progressive blunting of their crests. Finally, in the oldest experimental animal the septum between the first and second molars was completely destroyed. The interradicular septum of the first molar was markedly reduced in height, its crest was concave, and its marrow showed fibrosis and was invaded by inflammatory cells. These changes were associated with an interradicular pocket which was filled with a purulent exudate.

Thus, the sequence of changes occurring in the supporting structures of teeth of old mice were as follows: The administration of estrogen led to a proliferation of the epithelial attachment and the epithelium of the gingival papillae. The epithelial attachment first migrated along, and later became detached from, the root surfaces leading to the formation of periodontal pockets. As a result of pocket formation between the teeth and between the roots of teeth, the interdental and interradicular bony septa showed resorption.

Lansing and Opdyke[8] have shown that after fourteen days of stilbestrol treatment the epidermis of guinea pig nipples showed acanthosis, hyperkeratosis, and a threefold increase in the mitotic activity. Similar changes have been reported by Uehlinger, Jadassohn, and Fierz.[9] It has been shown in the female mice that during the proestrus period (estrogen phase) the mitotic activity and the thickness of the epidermis are considerably increased[10] and that during the diestrus period epithelial proliferation can be induced by estrogen injections.[11] Dunaif and Finerty[12] showed that ovariectomy resulted in a reduction in epidermal growth in rats and that injections of low doses of estrogen to ovariectomized rats prevented this lowering of growth rate. Ziskin, Blackberg, and Slanetz[5] reported an increase in hornification in estrogen-treated monkeys. The present investigation, in accord with previous findings, shows that administration of estrogen leads to the proliferation of gingival epithelium and the epithelial attachment.

(2) Response of Alveolar Bone to Estrogens.—Previous investigations on the skeleton of mice and rats have shown that estrogenic steroid hormones stimulate endosteal bone formation in long bones of mice but fail to do so in young growing rats.[1] It also has been previously established that the response of bone tissue to estrogens varies according to age. Whereas in mice 1 to 6 months old endosteal bone formation could be seen after about ten days of administration of estrogens, in aged mice (1 year or older) the osteogenic response

could not be noticed before eight weeks of treatment.[3] The findings of Stahl, Weinmann, Schour, and Budy[4] showed that administration of estrogens led to osteosclerosis in the alveolar process of young mice.

The present investigation, in accordance with previous findings, has shown that following the administration of estrogens the alveolar process of mice showed osteosclerosis, but that of the rats did not. The response of alveolar bone to estrogens was most marked in young growing mice and was absent in aged mice. The absence of response in the alveolar process of aged mice which were treated beyond eight weeks is not incompatible with the findings of Urist, Budy, and McLean.[3] These investigators have reported that after about eight weeks of treatment, comparable to that in this experiment, the proximal end of the tibia and distal end of the femur of old mice showed endosteal new bone formation. In the alveolar bone of aged mice in the present study, the presence of deep periodontal pockets and superseding inflammation led to the resorption of the alveolar process, and thus made a comparison of this region of the skeleton with other areas impossible.

(3) Age Changes Compared to Those Following Administration of Estrogens.—Although there are no reports in the literature which deal with age changes in the supporting structures of molars in the mouse, Belting, Schour, Weinmann, and Shepro[13] have reported such changes in the rat (Wistar albino). They have shown that in rats from 21 to 1,000 days of age the downgrowth of the epithelial attachment occured earlier and progressed farther on the mesial side of first molar than in any other area. In older animals where there was some food impaction between the molars, the downgrowth of epithelial attachment was not as great in the interdental areas as it was mesial to the first molar. Even in very old animals (900 to 1,000 days) which had severe food impaction, no pocket formation or proliferation of the gingival epithelium was noted. Also, it was observed that in cases where, due to age changes (900 to 1,000 days), the entire interdental septum was resorbed, the epithelial attachment remained high on the roots of the teeth. In the present study it has been shown that there was a marked proliferation of epithelial attachment in the interdental and interradicular areas, whereas on the mesial surface of the first molar no striking changes were seen. It has also been shown that there were deep interdental and interradicular pockets and that there was a marked proliferation of the gingival epithelium. The resorption of the alveolar septa and the downgrowth of epithelial attachment occurred simultaneously.

With regard to the interdental and interradicular bony septa, it has been reported[13] that the interdental septa decreased from 50 to 1,000 days of age by about 32 per cent of their width. This decrease in width was due to resorption on the mesial surface of interdental septa. No changes were noted in the interradicular septa. In the present study both the interdental and interradicular septa were progressively destroyed and disappeared at relatively younger age levels. Also, as evidenced by their concave upper surfaces, this resorption started from their crests and progressed apically.

Thus, the changes seen in the gingival epithelium, epithelial attachment, and alveolar bone of 1-year-old mice do not resemble those observed due to aging in 3-year-old rats. Also, since the 1-year-old control mice in the present experiment showed no changes similar to estrogen-treated animals, old age may be excluded as a cause for the observed periodontal disease.

SUMMARY AND CONCLUSIONS

This study is based on a radiographic and histologic analysis of the teeth, alveolar process, gingival epithelium, and epithelial attachment of groups of young rats, young mice, and old mice.

Sixteen newborn rats were divided into two groups, eight animals being used as controls and eight animals receiving a total of 0.05 to 1.30 mg. of a-estradiol benzoate. Animals were sacrificed from five to forty-two days after the start of the experiments.

Twenty-two newborn mice were divided into three groups, eight animals being used as controls, six being given a total of from 0.05 to 0.80 mg. of diethylstilbestrol and sacrificed at from 5 to 30 days of age, and eight mice receiving a total dosage of 0.05 to 1.30 mg. of a-estradiol benzoate and being sacrificed from 5 to 40 days of age.

Thirty-two 1-year-old mice were divided into three groups, six mice being used as controls, nine mice receiving a subcutaneous pellet of 2.0 mg. of crystalline a-estradiol benzoate and being sacrificed from one to thirteen weeks after the start of experiment, and eight mice receiving a total of 1.5 to 6.0 mg. of a-estradiol benzoate and being sacrificed from three to twelve weeks after the start of the experiments.

This investigation showed that:

1. The administration of estrogens did not lead to any changes in the teeth, alveolar process, gingival epithelium, or the epithelial attachment of the young rats.

2. In the newborn mice which received from 0.80 to 1.30 mg. of diethylstilbestrol or a-estradiol benzoate over a period of more than twenty-five days, there was a replacement of hemopoietic marrow of the alveolar process by a fibrous marrow and the marrow spaces were reduced in size by new bone formation.

3. In old mice which received injections or subcutaneous pellets of a-estradiol benzoate, there was an increased downgrowth of epithelial attachment along the root surface and into the bifurcation of roots. The gingival epithelium of the interdental papilla between the first and second molars also proliferated in an almost tumor-like fashion. The interdental papilla lost its conical shape and became progressively blunted and concave. In the final stages of the experiment there were deep interdental and interradicular pockets which then led to a marked resorption of the interdental and interradicular bony septa. The root surfaces showed many areas of resorption. The earliest of these changes were noted three weeks after the beginning of the experiment and they became progressively marked.

TABLE I

SUMMARY OF RADIOGRAPHIC AND HISTOLOGIC CHANGES IN THE GINGIVA AND ALVEOLAR BONE OF RATS AND MICE FOLLOWING ADMINISTRATION OF ESTROGEN

ANIMAL GROUP	AGE AT START OF EXPERIMENT	DURATION OF EXPERIMENT	EXPERIMENT	RADIOGRAPHIC FINDINGS	HISTOLOGIC FINDINGS			
					ALVEOLAR BONE	EPITHELIAL ATTACHMENT	GINGIVAL EPITHELIUM	TEETH
Young rats	0 days	5 to 42 days	0.05 to 1.30 mg. a-estradiol benzoate	No changes	No changes	No changes	No changes	No changes
Young mice	0 days	5 to 25 days	0.05 to 0.30 mg. diethylstilbestrol or a-estradiol benzoate	No changes	No changes	No changes	No changes	No changes
Young mice	0 days	25 to 40 days	0.80 to 1.30 mg. diethylstilbestrol or a-estradiol benzoate	Absence of marrow spaces in interradicular and interdental septa	Marrow spaces reduced in size and number; endosteal bone formation; fibrous marrow	No changes	No changes	No changes
Old mice	1 year	1 to 13 weeks	Subcutaneous implant of 2.0 mg. of a-estradiol benzoate	Widening of PDM; resorption of interdental and interradicular bony septa	No changes	Downgrowth of epithelial attachment both in interdental and interradicular areas; pocket formation	Inflammatory cells in the epithelium; downgrowth of the gingival epithelium; interdental papilla concave and blunt	Resorption of root surfaces
Old mice	1 year	3 to 12 weeks	1.5 to 6.0 mg. a-estradiol benzoate	From slight widening of PDM to partial or complete destruction of interdental and interradicular septa	Resorption of interdental and interradicular bony septa	Downgrowth of epithelial attachment in interdental and interradicular areas; pocket formation; exposure of root surfaces; deep pocket formation with purulent exudate	Proliferation of gingival epithelium	Resorption of root surfaces

The radiographic and histologic changes in the gingiva and the alveolar bone of rats and mice following the administration of estrogens are summarizd in Table I.

The help given by Mr. William M. Winn, biologic photographer, in the preparation of the photomicrographs is gratefully acknowledged.

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THE LOCALIZATION AND INTERRELATION OF NUCLEIC ACIDS AND ALKALINE PHOSPHATASE IN THE DEVELOPING TOOTH

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INTRODUCTION

THE increasing interest and apparent importance of nucleic acids in numerous metabolic events has prompted us to undertake a study of the cytological localization of these substances in developing teeth. We are particularly interested in nucleic acid relationships occurring in those cells or tissues intimately concerned with the elaboration of mineral salts or the organic matrix. We are further interested in establishing such correlations as may exist between the presence of nucleic acids and other cystochemical components of these cells.

The studies of Caspersson[6] and Brachet[3, 4] have resulted in the identification of nucleic acids in a variety of cells. These authors have emphasized the role of nucleic acids in the elaboration of proteins. Likewise, the possibility that nucleic acids may be concerned with the process of calcification appears to have occurred to previous investigators. Thorell and Wilton[18] reported a reduction in nucleotides in the odontoblasts as these cells become morphologically differentiated. They also observed that odontoblasts of scorbutic animals revert to a functional stage as regards nucleic acid metabolism similar to that which occurs in the young undifferentiated odontoblast. Thorell[19] referred to an apparent inverse correlation between the disappearance of polynucleotides in the differentiating odontoblasts coupled with the advent of calcification of dentin, based on Engström's analysis.[9] Cappellin[5] noted a reduction of the cytoplasmic nucleic acid in osteoblasts of the developing chick bone, correlated with the deposition of osseous material. Wislocki and Sognnaes[20] also reported the presence of basophilia attributable to ribonucleoprotein in the cytoplasm of odontoblasts and ameloblasts in growing teeth. Follis,[10] from a study of scorbutic animals, concluded that the presence of ribonucleic acid (RNA) in the cytoplasm of the osteoblast is significant, and suggested the possible relation between the presence of RNA and the production of proteins. Pritchard[14] described a progressive increase in RNA content in differentiating osteoblasts, while a decided loss in cytoplasmic basophilia was evident in osteocytes. This author also stated that osteogenic cells exhibiting a maximum amount of cytoplasmic RNA are coupled with the sites of most rapid deposition of bone matrix. Rosin,[15] in a study of fracture healing, also

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described the coexistence of phosphatase and ribonucleic acid in cartilage cells and osteoblasts in the calcifying tissues associated with the healing process.

It is thus apparent from this survey that nucleic acids have been reported in osteogenic and dentinogenic cells. The detailed description of the localization of both desoxyribonucleic acid (DNA) and RNA are still to a large degree fragmentary. It is further clear that the implications drawn by the various investigators who have studied the localization of nucleic acids in calcifying tissues, while suggestive of the possible role they may play in the development of these tissues, are but speculative in nature. In any event, the correlation between the presence of nucleic acids and their definitive metabolic role has by no means been established.

MATERIALS AND METHODS

The material used in this study consisted of developing incisor teeth of pig embryos obtained from freshly slaughtered animals. Specimens were immediately fixed in Carnoy's solution according to the usual methods used for studying the localization of nucleic acids. Other specimens were fixed in 95 per cent ethyl alcohol and Helly's fluid. Tissues were embedded in paraffin and cut 7 μ. The methyl-green-pyronin stain was used according to the methods of Pollister and Leuchtenberg,[13] Taft,[17] and Kurnick.[12] The pyronin Y used was a sample certified in 1952 by the Commission on Biological Stains and was found to give consistently reproducible results. The methyl-green employed was repeatedly extracted with chloroform before using. Removal of ribonucleic acid was accomplished by digestion of tissues in a solution of ribonuclease (Worthington) in distilled water. Extraction also resulted following treatment with perchloric acid.[16]

Desoxyribonucleic acid was identified by the Feulgen method and also by the use of methyl-green staining preceded by digestion with desoxyribonuclease (Worthington). Alkaline phosphatase was identified by the Gomori method.[11] The basophilic properties of the tissues were ascertained by the use of N/2000 methylene blue at pH 6.6 which, after testing various pH values, was found to yield the most satisfactory results.

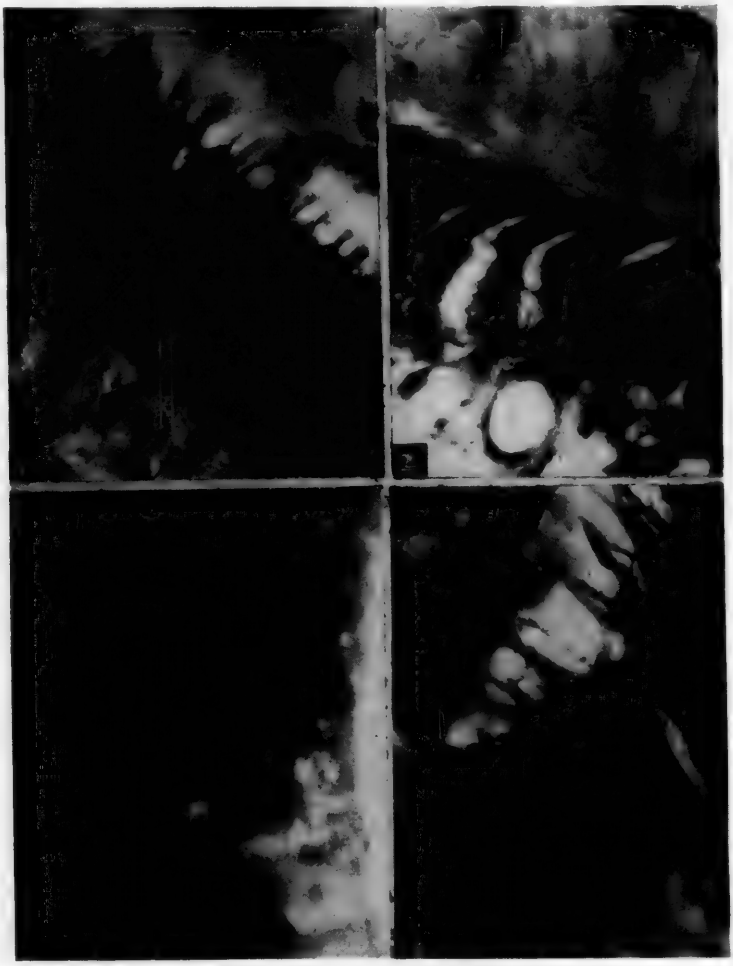
OBSERVATIONS

Nucleic Acid.—

A. *Ribonucleic acid:* The following observations are based on examination of sections of developing teeth obtained from embryos ranging from 120 mm. to 170 mm. The state of differentiation included in this span reveals the diverse cells of the developing tooth in practically all stages of differentiation, including functionally mature odontoblasts, ameloblasts, and increments of calcified enamel and dentin.

Sections stained with methyl-green-pyronin show a weak pyroninophilia in the cells of the stellate reticulum, in the fibers of the dental sac, the cytoplasm of the cells making up the stratum intermedium, and in the cells of the dental pulp. Examination of the ameloblastic layer shows that as the amelo-

blast develops, the cytoplasm gradually acquires an affinity for pyronin. The pyroninophilic material in the differentiating ameloblast is localized in the infranuclear region of the cell and appears as granular material. As the ameloblast elongates and comes to be contiguous with enamel, the pyroninophilic material in the ameloblast appears much more extensive in its distribution than in the younger cells. In this functional stage the pyroninophilia is most pronounced in the densely stained rodlike structures located in both a



Figs. 1 through 10 are photographs of sections of the lower incisor of 170 mm. specimens.

Fig. 1.—Ameloblasts stained with methyl-green-pyronin showing intense staining of rodlike structures in the cytoplasm and also pyroninophilic nucleoli.

Fig. 2.—Odontoblasts stained with methyl-green-pyronin showing intense pyroninophilia of cytoplasm and nucleoli.

Figs. 3 and 4.—Sections showing localization of alkaline phosphatase in ameloblasts and odontoblasts, respectively, which were incubated in a substrate containing adenosine-3-phosphate.

supra- and infranuclear position (Fig. 1). Tome's enamel processes are also faintly pyroninophilic, as is likewise the enamel matrix.

Odontoblasts likewise gradually acquire an affinity for pyronin as the cells differentiate. In the apical region of the developing tooth these cells show only a mild affinity for pyronin, while in the area where the increment of dentin begins to calcify, the cytoplasm gradually becomes more intensely pyroninophilic (Fig. 2). In the younger or less differentiated cells the pyronin appears to be localized in granular form; as the cell matures the cytoplasm of the odontoblast appears to be uniformly stained by pyronin. Another characteristic cytological feature which the odontoblasts reveal following pyronin staining is the presence of a number of very delicate basophilic fibrils distributed throughout the cytoplasm of the cell. Tome's dentinal processes and developing dentin are also stained slightly with pyronin.

In order to ascertain the localization of ribonucleic acid in the cells of the developing tooth, a number of sections similar to those just described were treated with ribonuclease or with perchloric acid. Following either of these treatments the sections were then stained with methyl-green-pyronin. Examination of sections shows that, with the exception of the odontoblasts and the ameloblasts, all of the cellular constituents we have described as staining with pyronin show a mild reduction in pyroninophilia. The ameloblasts and odontoblasts as shown in Figs. 5 and 6 fail to stain with pyronin. Our observations indicate that the cellular components of the stellate reticulum and the pulp contain some ribonucleic acid. The ameloblasts and odontoblasts gradually acquire a considerable amount of cytoplasmic RNA as the cell differentiates. During odontogenesis and amelogenesis the cytoplasmic RNA appears to be abundant.

B. *Desoxyribonucleic acid:* Detailed examination for nucleic acid in the nuclei of the various cells of the developing tooth was confined for the most part to the odontoblasts and the ameloblasts. In the odontoblasts the desoxyribonucleic acid content of the nucleus appears to increase as the cell differentiates, and reaches a maximum at approximately the time at which calcification of dentin begins. The nuclei of more mature odontoblasts appear to become somewhat reduced in size and in RNA content. The nuclei of the ameloblasts gradually enlarge as the cell differentiates. We observe a gradual increase in the DNA content in the nuclei of these cells which appears to be maintained at a relatively constant level during the functional period under which they were observed. The nuclei in both the odontoblasts and the ameloblasts contain RNA localized in the nucleoli. In the odontoblast the nucleolus diminishes in size as the cell becomes functional; the nucleolus of the ameloblast appears to reach a maximum size as the cells become functional and to remain relatively unchanged during the remainder of the functional state of these cells during which time enamel is elaborated.

Basophilia.—In connection with the histochemical tests utilized to localize nucleic acids a number of sections were also stained with dyes such as methylene blue in order to compare the basophilic elements thus produced with those

obtained with methyl-green-pyronin. Our interest in the basophilia exhibited by the structural components of the developing tooth is twofold. First, it is well known that dyes such as methylene blue stain nucleic acids. Second,

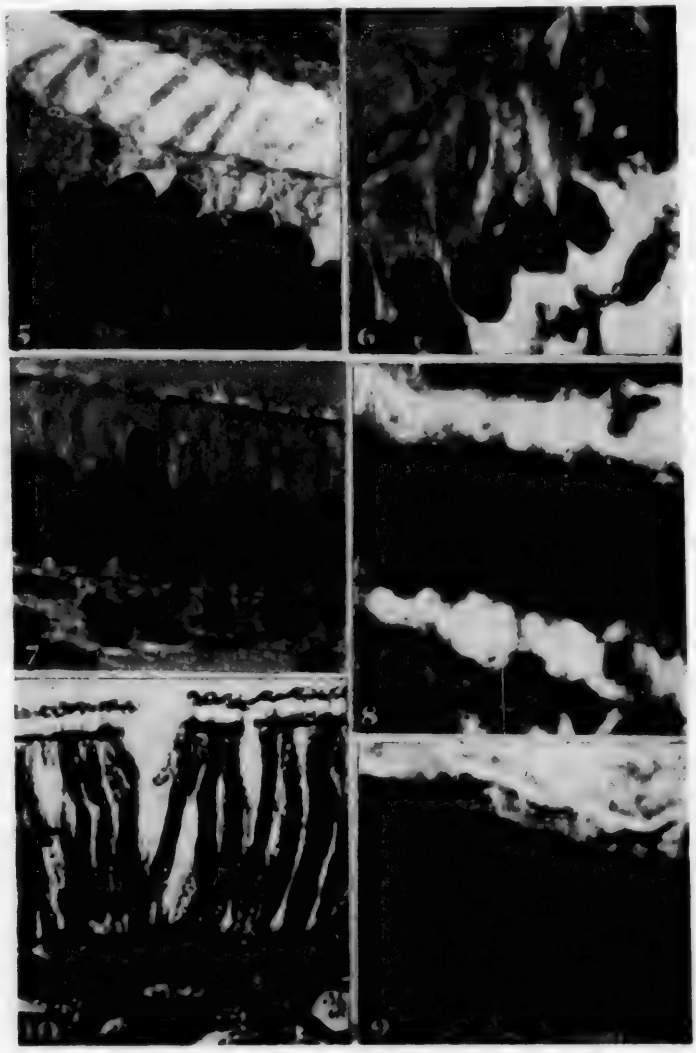


Fig. 5.—Ameloblasts digested with ribonuclease and subsequently stained with methyl-green-pyronin. Note absence of pyroninophilia in ameloblastic cytoplasm and Tome's processes. (Compare with Fig. 1.)

Fig. 6.—Odontoblasts digested with ribonuclease and subsequently stained with methyl-green-pyronin. Note absence of cytoplasmic pyroninophilia. (Compare with Fig. 2.)

Fig. 7.—Ameloblasts stained by the Feulgen method. (Compare with Fig. 1.)

Figs. 8 and 9.—Odontoblasts and ameloblasts stained with methylene blue to show basophilia.

Fig. 10.—Section showing localization of alkaline phosphatase in ameloblasts which were incubated in a substrate containing nucleic acid.

they also stain cytochemical constituents other than nucleic acids. Odontoblasts and ameloblasts exhibit a marked basophilia as shown in Figs. 8 and 9. Comparison of these methylene blue preparations with those stained with methyl-green-pyronin shows that the basophilia exhibited by these cells is more diffuse than the pyroninophilia. Sections incubated in a solution containing ribonuclease and subsequently stained with methylene blue indicate that most, but not all, of the cytoplasmic basophilia is removed. The cytoplasm of the odontoblasts and the ameloblasts has the optical appearance illustrated in Figs. 6 and 7 still exhibiting a mild basophilia. The results of the tests on the basophilic properties of these cells indicate that the presence of nucleic acids in ameloblasts and odontoblasts substantiates the results obtained by the methyl-green-pyronin method. Of equal interest is the identification of other (residual) basophilic cytochemical constituents in these cells.

Phosphatase.—Inasmuch as we were interested not only in the identification of specific cytochemical constituents such as nucleic acids in the tooth forming cells but also in the interrelationships of other cytochemical constituents, we re-examined the localization of alkaline phosphatases with this thought in mind. We extended our previous observations[1, 2] to include yeast nucleic acid and adenosine-3-phosphate as components of the incubating solutions by which the localization of phosphatase is ascertained through enzymatic hydrolysis of phosphate ions.

The results of the tests conducted with nucleic acid and adenosine-3-phosphate are shown in part in Figs. 3, 4, and 10. Examination of these preparations shows (1) that through the use of either of these substrates phosphatase can be demonstrated in appreciable amounts in functional ameloblasts and odontoblasts; and (2) that while there appears to be a difference in the intensity of the phosphatase reaction with different substrates, in the ameloblasts and the odontoblasts there is no apparent difference in the pattern of localization of the enzyme when tested with the two substrates we have just mentioned or any of the several other substrates we have previously tested.

DISCUSSION

We have demonstrated by histochemical methods that both ribo- and desoxyribonucleic acids are conspicuous components of the ameloblasts and odontoblasts of the developing tooth. We also observe that nucleic acids in these cells gradually appear as the cell differentiates and reach a relatively high level which is maintained during the active functional period, during which time the calcified tissues are being elaborated. We have also demonstrated in this and previous studies that the time of appearance and the sites of localization of the enzyme alkaline phosphatase in these two cells coincide to a remarkable degree. Similar observations have been reported to obtain in osteoblasts by Pritchard,[14] Rosin,[15] and other investigators.

Dempsey and Wislocki[8] report several examples of either the coexistence or an inverse relationship between ribonucleic acid and phosphatase. In this connection, they suggest that phosphatase may be implicated in nucleoprotein

and nucleotide metabolism. They further suggest that phosphatase may serve as a dephosphorylating enzyme acting upon nucleic acids or, conversely, phosphatase may provide phosphate ions which are utilized in the synthesis of nucleotides.

In the present studies, we have demonstrated the coexistence of nucleic acids and alkaline phosphatase in odontoblasts and ameloblasts. In the ameloblasts one can readily establish a direct correlation between the presence of ribonucleic acid, phosphatase, and production of the protein matrix of enamel. While a coexistence of nucleic acids and phosphatase has also been established in the odontoblasts, we cannot at this time establish a direct correlation between the presence of these substances and the production of extracellular protein, as is true in the ameloblasts.

One of the well-known functions of nucleic acids has been shown to be concerned with the internal economy of the cell. In addition, several other phenomena such as protein synthesis, phosphate donation, evocation in embryological development, inhibition or synthesis of enzyme systems have also been related to the presence of nucleic acids in cells.[7] In this connection, we have demonstrated the presence of nucleic acids in the ameloblast and odontoblast during the time in which enamel and dentin are being elaborated, and also the fact that ribonucleic acid and phosphatase coexist in these cells during this process. The presence and interrelation of both phosphatase and nucleic acids in cells known to be intimately associated with the formation of calcified tissues suggest that they are involved in the elaboration of dentin and enamel. The specific role which these substances play in the elaboration of these tissues can only be speculated on at the present time; additional research is needed to extend our knowledge of the factors which initiate and govern the growth of calcified tissues.

SUMMARY

A histochemical study has been undertaken with special reference to the localization of nucleic acids in the developing tooth. We have shown that the odontoblasts and ameloblasts acquire considerable amounts of cytoplasmic RNA in the period during which dentin and enamel are elaborated. We have also shown that RNA and alkaline phosphatase coexist in terms of time and localization in these cells. While we cannot draw any definite conclusions as to the role these two substances may play in the process of calcification, it appears from our observations that the possibility that nucleic acid is implicated in some phase of the calcifying mechanism must be considered as a likely possibility.

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STAINING REACTIONS FOLLOWING DEMINERALIZATION OF HARD TISSUES BY CHELATING AND OTHER DECALCIFYING AGENTS

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ALL the usual methods for demineralization of hard tissues require the use of solutions with low pH's, with the exception of the method recommended by Kramer and Shipley[2] using magnesium citrate.

Because of the limited number of techniques available for decalcifying hard tissues at alkaline pH's, the present study was planned to observe the effects of using one of the chelating agents, the sodium salt of ethylenediaminetetraäcetic acid,* as a decalcifying medium, and to compare the effects with those of conventional acidulated solutions on the staining reactions of dental and osseous tissues following the use of hematoxylin and eosin.

It is recognized that the procedures employed in histopathologic techniques are based as much on empiricism as on known scientific methods, and that the differences in color values and clarity of cytologic detail of different sections are necessarily assessed subjectively.

METHOD

The following experiments were designed to determine the influence on staining reactions of different concentrations, pH's, and temperatures of the chelating reagent, of different decalcifying solutions, and of different fixatives, using rat and guinea pig femurs and mandibles.

1. *Concentration Series.*—Six rat mandibles were decalcified in EDT solutions at 25° C. and at a pH of 7.40 to 7.45, the variable being the concentration of the solution used, that is, 0.2 M to 0.7 M, increasing by 0.1 molar increments.

2. *pH Series.*—Four rat femurs were decalcified in EDT solutions having a constant concentration of 0.25 M, but with the pH varied as follows: (a) 6.0, (b) 7.5, (c) 9.5, and (d) 12.0.

3. *Temperature Series.*—Four guinea pig femurs were decalcified in 0.5 M EDT solutions at a pH of 7.5. The temperature of the solutions varied as follows: (a) 4° C., (b) 25° C., (c) 37.5° C., and (d) 60° C.

4. *Decalcifier Series.*—Seven guinea pig femurs were decalcified at 25° C. in the following solutions: (a) 5 per cent nitric acid, (b) 45 per cent formic acid and 20 per cent sodium citrate,[4] (c) 45 per cent formic acid and surplus resin (Amberlite),† (d) 45 per cent formic acid and surplus resin (Win-

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*The ethylenediaminetetraäcetic acid used in this experiment was obtained from the Bersworth Chemical Co., Framingham, Mass., under the trade name Versene. The material will be referred to in the text by the abbreviation EDT. The proper pH's were obtained by neutralizing the sodium salt with hydrochloric acid.

†Amberlite 1RC-50 (h) manufactured by Rohm & Haas.

3000),* (e) magnesium citrate solution as prepared by Kramer and Shipley,[2] (f) 5 per cent nitric acid with 1 per cent phloroglucin,[3] and (g) 0.5 M EDT solution at pH 7.5.

5. *Fixative Series.*—The influence of the fixative medium on the staining reaction was observed by using two sets of four guinea pig femurs decalcified identically in EDT solutions (0.5 M at 25° C.), but with varying pH's, namely (a) 6.1, (b) 7.5, (c) 9.5, and (d) 11.8. One set was fixed for forty-eight hours in 10 per cent neutral formalin (pH 7) and the other in Zenker-formol solution for forty-eight hours. Other sets, fixed in the same way, were decalcified in 5 per cent nitric acid, 45 per cent formic acid with resin, magnesium citrate, 5 per cent nitric acid with 1 per cent phloroglucin, and 45 per cent formic acid with equal parts of 20 per cent sodium citrate.

All of these tissues were embedded in celloidin (Parlodion) and sectioned at 10 μ. Sections were mounted on slides, decelloidinized, and stained together in the same batch of stain (Harris' hematoxylin and eosin Y).

RESULTS

1. *Concentration Series.*—The stronger the solution of EDT, the shorter was the time of decalcification. The time ranged from eight to thirteen days. The staining reactions of all six specimens were indistinguishable. The staining was satisfactory in intensity and clarity.

2. *pH Series.*—All four specimens exhibited similar staining reactions, and these were well differentiated and gave good nuclear detail. The rate of decalcification was retarded as the pH was increased as follows: (a) eight days, (b) nine days, (c) sixteen days, and (d) eighteen days.

3. *Temperature Series.*—The temperature of the EDT solutions affected the time taken to decalcify the guinea pig femurs. At 4° C., decalcification required seven days; at 23° C., it required six days; at 37.5° C., three days; and at 60° C., it required only one day. As a group they stained similarly.

4. *Decalcifier Series.*—The time taken by each of the seven solutions to decalcify a guinea pig femur was as follows.

(a) 5 per cent nitric acid	one day
(b) 45 per cent formic acid and 20 per cent sodium citrate	three days
(c) 45 per cent formic acid and resin (Amberlite)	one day
(d) 45 per cent formic acid and resin (Win-3000)	one day
(e) Magnesium citrate at pH 7.5	thirty days
(f) 5 per cent nitric acid and 1 per cent phloroglucin	two days
(g) EDT (0.5 M at pH 7.5)	six days

This result clearly showed that the acid solutions were quicker than the alkaline solutions in decalcifying tissues, but that, of the latter, the chelating agent is much faster than the magnesium citrate method.

In the matter of staining reaction, specimens decalcified with nitric acid were not as clear in detail as those decalcified in EDT, nor did they absorb as much eosin stain. The formic acid-sodium citrate solution produced results that were indistinguishable from those produced using EDT, whereas

*Win-3000 obtained from Winthrop-Stearns, Inc.

the formic acid and resin specimens were more deeply stained. The clarity using the latter two methods was comparable to that using nitric acid, but poorer than that of EDT. This may, however, be due to individual tissue variation in this experiment. Tissues decalcified with magnesium citrate gave staining results that had noticeably too much hematoxylin in the nuclei, but the eosin content was quite satisfactory for differentiation and detail. The sections obtained after using nitric acid with 1 per cent phloroglucin were by far the poorest in nuclear stain. Very little hematoxylin was present in the cell nuclei, although bone matrix took up a fair amount of eosin. The sections were otherwise foggy, and the cellular detail was very inadequate.

5. *Fixative Series.*—No differences could be detected in the tissues decalcified in EDT at various pH's and fixed in formalin. The four Zenker-formol-fixed tissues also stained uniformly. There was, however, a constant difference between the sets in that the formalin-fixed tissues showed more eosin than did the Zenker-formol-fixed tissues. Also, the Zenker-formol group showed a denser nuclear stain than did those fixed in formalin. This difference in group staining was observed also in the formic acid-sodium citrate solution. The magnesium citrate decalcified tissues showed the same denser nuclear stain, but similar staining of the cytoplasm with eosin, and two of the solutions did not show these differences, that is, 5 per cent nitric acid and formic acid with resin. Both of these latter two sets stained similarly, although too heavily. As mentioned before, the tissues exposed to phloroglucin stained very poorly.

CONCLUSIONS

It has been shown that when using the alkaline chelating agent EDT as a tissue decalcifier the following facts were observed to apply within the experimental limits detailed previously.

1. The concentration of the solution has no effect on staining.
2. The temperature of the solution has no effect on staining.
3. The pH of the solution has no effect on staining.
4. The staining results obtained were the equal of and in some instances better than those obtained when using conventional acid solutions.

This suggests that the chelating agent EDT is a satisfactory decalcifying medium, suitable for routine histologic staining over a wide range of physical conditions.

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AN APPARATUS AND A TECHNIC FOR THE PREPARATION OF GROUND SECTIONS AND THE PERFORMANCE OF SERIAL SURFACE GRINDINGS

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INVESTIGATIONS of undecalcified tissues by direct illumination demand an accurate method of grinding down the slabs into thin transparent sections with planoparallel surfaces. Investigations by incident light also require planoparallel grindings, especially if this technic is used to gain a three-dimensional conception of the tissue in question. In that case, the object could be examined through a series of planoparallel surfaces with a predetermined distance between them.

To meet these needs at our laboratory, a simple grinding apparatus was designed and made to order. The intention was to combine some of the advantages of other machines constructed to prepare ground sections, and thus to obtain an apparatus with a satisfactory capacity at the lowest possible cost.

A brief technical description of the apparatus and its use, including a review of the plastic embedding and the mounting technic employed, is given in this article.

GRINDING APPARATUS

To save space and facilitate transport, the motor and unit for advancing the specimen during grinding are mounted on a common base plate. All transmission of power has been avoided, the grinding wheels being mounted directly on the motor shaft.

The motor, which is an "Indola" Voorburg Z.H., type K.V.B., has the relatively low speed of approximately 800 r.p.m., and has sufficient power for this special purpose, provided the appropriate grade of abrasive is used.

The main part of the advance unit (Fig. 1) is the guide shaft, *A*, a cylindrical steel rod carrying the removable object disk, *B*, and the carrier, a brass tube, *C*, through which the guide shaft may be advanced evenly and steadily as the grinding proceeds. Rotation of the shaft is prevented by a steel rail screwed to it and fitting into a longitudinal groove in the carrier.

Grinding pressure is exerted by the tension of a spring acting on the guide shaft by means of a spring rod, *D*, and the holding arm, *E*, of the latter. The spring tension may be controlled by the adjustable spring stop on the rod, *F*. The holding arm is kept in adjustable position on the guide shaft by

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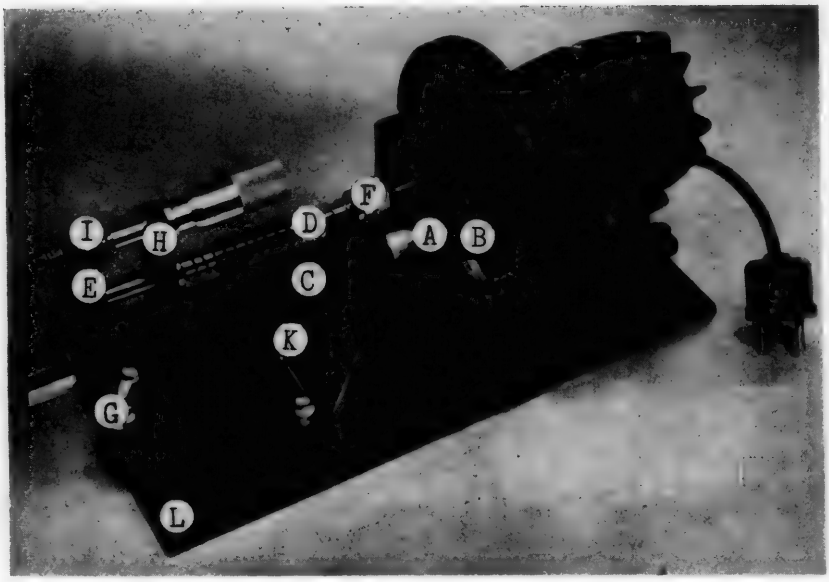

Fig. 1.—Apparatus used in the preparation of ground sections and in surface grinding for microscopic study. *A*, Guide shaft; *B*, object disk; *C*, carrier; *D*, spring rod; *E*, holding arm of spring rod; *F*, spring stop; *G*, set screw for fixing position of holding arm; *H*, holding arm of micrometer check-screw; *I*, insulated stopper; *K*, set screw for advance unit; *L*, main base plate.

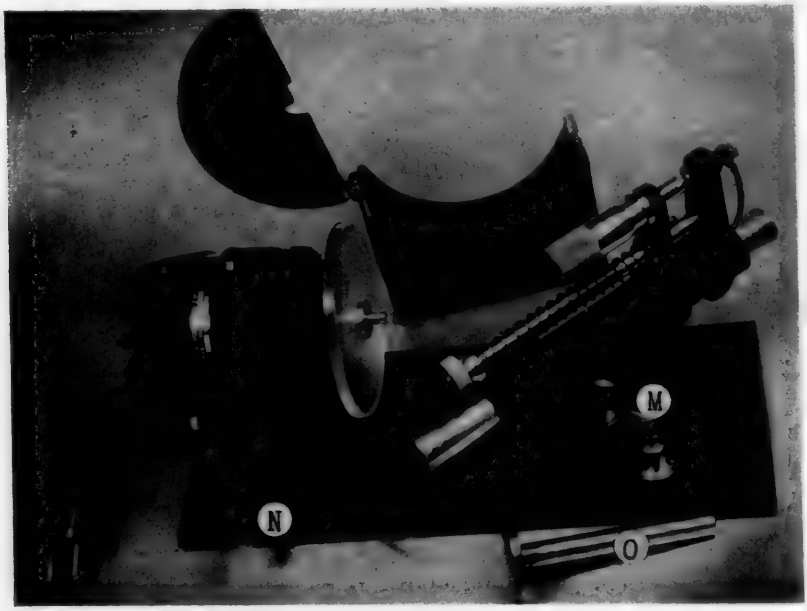

Fig. 2.—Apparatus shown in Fig. 1. *M*, Spring clamp securing grinding position of advance unit; *N*, signal lamp; *O*, accessory handle for manipulation of the object disk.

a set screw, *G*. Thus, it is possible to vary the distance between the object disk and the grinding wheel without having to readjust the micrometer check-screw and the spring stop. The arrangement is convenient for serial grindings of specimens in toto embedded in tall columns of plastic.

To the carrier is attached the holding arm, *H*, of the micrometer check-screw, *H'*. When grinding is completed according to the adjustment of the check-screw, the latter contacts the stopper, *I*, in the head of the holding arm of the spring rod. The stopper is insulated from the metal of the arm, and contact thus may be signaled by a lamp, *N*, supplied with current through the micrometer check-screw and the stopper.

The carrier is mounted on a circular steel base plate, in the center of which there is a solid set screw, *K*, connecting it with the main base plate (Fig. 2).

The advance unit may be revolved on the set screw to reveal the object disk, and it is kept in exact and steady grinding position by a spring clamp, *M*, fitting into a groove drilled in the main base plate, *L*.

EMBEDDING TECHNIC

Successful use of the apparatus is chiefly dependent on an appropriate application of the plastic embedding and mounting technic described by Puckett[5] and Sognnaes.[6] A few details of this technic have been modified, however, on account of some difficulties in obtaining the proper chemical substances required at various stages of the procedure.

Instead of pure benzoyl peroxide which is generally used to accelerate the hardening of a monomer liquid, the polymer powder of clear plastic, applied in dental practice to accelerate the polymerization of acrylic dentures, was used at our laboratory as a catalyst.

The powder is added to the monomer in the proportion of 1 to 4 or 5 parts of liquid by volume. The sample, kept in a flask, is stirred until the powder particles no longer sink to the bottom when stirring or shaking is interrupted. The stirring must continue for at least one-quarter of an hour. By the end of this time the sample has acquired a viscous consistency like syrup, and has become milky. To clarify, it is left standing for twenty-four hours before use. The hardening time of this embedding medium and its tendency to bubble formation are factors dependent not only on the temperature to which it is exposed, but also on the kind and size of the specimen that it contains. Even the containers in which the polymerization takes place influence the rate of the process. For instance, when small glass tubes were used the polymerization process was completed without bubble formation within only twelve hours, while the process taking place in rigid paper containers of the same size might take seven days for completion. In both cases the specimens embedded were human molars, and the temperature in the thermostat where the containers were placed was kept constant at 45° C.

It is probable that these effects are due to the conduction and distribution of the exothermic heat produced by the methyl methacrylate during hardening.

CUTTING AND GRINDING OF SECTIONS

The end product of polymerization, the hard plastic block, is cut into wafers, each framing a slab of the specimen. These in turn are mounted on plastic slides before grinding.

The construction of the cutting machine used at our laboratory and also the cutting procedure have been taken from the description given by Patten and Chase.[4]

In attaching the wafers to the slides, chloroform may be used as a dissolving agent instead of ethylene dichloride. One small drop of chloroform is conveyed to the center of the slide and the wafer is pressed firmly against the drop and kept under strong pressure by means of spring clips for one-quarter of an hour.

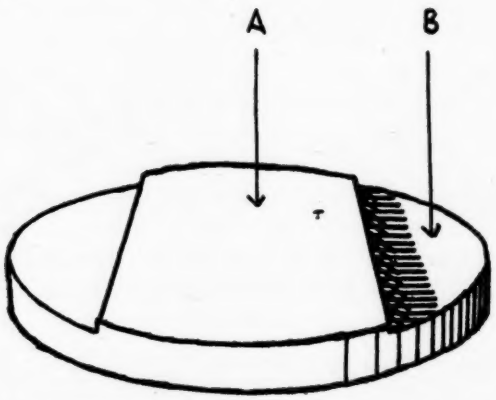

Fig. 3.—The object disk. *A*, Platform; *B*, depression for binding medium. The depth of the depressions has been exaggerated for the sake of clearness.

Before the slide can be attached to the object disk, the zero point on the check-screw must be read off; that is, the contact point between the stopper and the check-screw when the face of the object disk contacts the grinding wheel. To the number thus read is added the thickness of the slide and the desired thickness of the ground section. The check-screw is then adjusted to stop the advance of the guide shaft at the number calculated in this way. Repetition of this procedure is necessary only to compensate for wearing of the grinding wheel, or when the position of the holding arm of the spring rod has been altered.

To fasten the slide to the object disk, viscous Canada balsam may be employed. However, to avoid the trouble of calculating the thickness of a layer of Canada balsam between the slide and the disk, the latter has been shaped so as to form a plane platform, upon which the slide may rest directly, and two very shallow depressions on either side, to which the binding medium is applied after the disk has been heated over a Bunsen burner (Fig. 3).

The grinding wheels used were 5 mm.-thick plane brass disks. The abrasive was powdered carborundum suspended in water, and was fed to the wheel by a small brush during operation.

It is necessary to consider the thickness of the abrasive when adjusting the check-screw. For powdered carborundum of 400 grit, this number approximated 20 microns.

To remove carborundum particles from the surface of the ground section, soft rubbing with a wet cloth dipped in powdered pumice proved very effective.

The grinding pressure was very low—no more than was necessary to advance the guide shaft. Thus, it was possible to grind sections down to 80-50 microns without fracturing or loosing parts during the operation, which for one section was completed within a couple of minutes.

The ground sections, when cleaned and dried, are covered with a drop of Canada balsam and an ordinary cover glass.

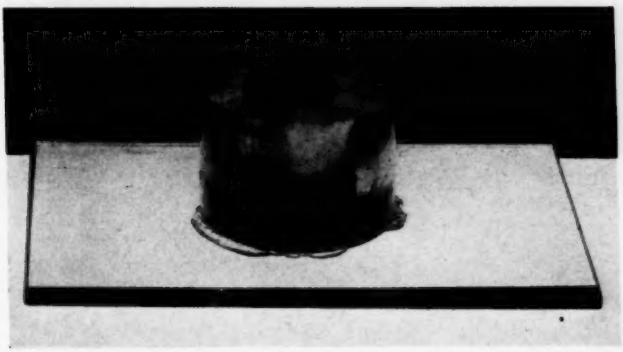

Fig. 4.—A plastic column containing a tooth, mounted on plastic slide.

SURFACE GRINDINGS

One of the main causes of the inferiority of any method of preparing ground sections, as compared with the ordinary microtome sectioning of decalcified tissues, is that whole sections are lost by the sawing up of the specimen. In practice there is also a lower limit to the thickness of such slabs when cut by a rotating disk.

In investigations of undecalcified teeth, this disadvantage may be overcome if microscopical examination by incident light is applicable in the particular case. Then the distance between the ground surfaces may be only 50 to 20 microns.

The plastic column containing the specimen is mounted on a slide by means of chloroform. The slide in turn may be fastened to the object disk in the manner just described (Fig. 4).

The grinding procedure is much simpler, however. The guide shaft is advanced until the plastic column contacts the grinding wheel. The figure for this position is read off on the check-screw, which is then turned to the check point that determines the thickness of the layer to be ground away.

After examination of the ground surface, the column may be replaced on the object disk for further grinding. It is thus possible to investigate a specimen through a continuous series of planoparallel surfaces.

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